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Contents

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Genetic Study on Retinits Pigmentosa ..... *Fumiko Kobayashi*

Studies on the Theoretical Values of Lens Flexibility ..... *Ryoichi Yamaji*

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13

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Contents

Adult Ocular Toxoplasmosis : Report of a Case of Central Exudative Retinitis due to Toxoplasmosis ..... *Hiroshi Ikui, Masabumi Nonaka, Ichiro Miyazaki, and Itsuo Yamamoto*... 77

Genetic Study on Retinitis Pigmentosa ..... *Fumiko Kobayashi*... 82

Studies on the Theoretical Values of Lens Flexibility ..... *Ryoichi Yamaji*... 88

Studies on Adenovirus Type 11 Infection of the Eye ..... *Shigeyuki Koseki*... 92

Experimental Pathological Studies on the Difference and Similarity between Trachoma Granules and Nontrachomatous Follicles ..... *Kazuo Hemmi*... 99

Histological Studies on the Ocular Toxicity of Alpha-Chymotrypsin ..... *Kunio Sekiguchi*...104

Studies on the Ribofravin Metabolism in Epidemic Keratoconjunctivitis ..... *Seishi Shinozuka*...117

Studies on the Nicotinic Acid Metabolism at Various Eye Diseases ..... *Tutomu Motegi*...128

Study on the Crystalline Lens by Polarized Light ..... *Nobuhiro Okada*...137

ADULT OCULAR TOXOPLASMOSIS

REPORT OF A CASE OF CENTRAL EXUDATIVE RETINITIS DUE TO TOXOPLASMOSIS

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The significance of toxoplasmosis as a cause of retinal lesions and uveitis is fully recognized in Europe and America, and it is said that an ocular lesion is not infrequently the only clue to the clinical diagnosis of toxoplasmosis. In Japan, however, no actual case of toxoplasmosis has been noted by any author among patients with any form of ocular diseases, probably because there has been no thorough-going parasitological investigation made as yet in the ophthalmological field, and not because there has existed no actual case of this disease. Our recent systematic examination, in the skin test by toxoplasmin and in the dye test of Sabin and Feldman, of a large number of patients with ocular diseases has shown that toxoplasmosis is identifiable in no small proportion of such patients. The one probable case of toxoplasmosis to be described in this paper is an adult patient with central retinitis: the macular region of the retina in his right eye was marked with the presence of a distinct exudate and bore a striking resemblance to the typical figure of toxoplasmic central chorio-retinitis as described by many authors; his cerebrospinal fluid contained the *Toxoplasma* in its separable form; these facts suggested that central retinitis in this patient was caused by infection with this protozoan in all probability. A brief description will be given, besides, of the progress so far made in the study of toxoplasmosis in Japan.

Report of a Case

M.K., a 29-year-old male, a chauffeur. He was first examined in the Ophthalmological Clinie of Kyushu University on March 26, 1958. In the middle of August, 1957, he had noticed blurred vision and metamorphopsia in his right eye, but no micropsia. The blurred vision, left untreated, aggravated later till the eye began to give the patient an impression that it was coated in front with a membrane. On February 21, 1958, the diagnosis of central retinitis of the right eye was made at a certain hospital; V.D. 20/40, V.S. 20/15. Systemic treatment with salicylate and iodine preparations was done at the hospital, but not effective in the disease. The vision in the right eye was reduced to 20/400 on March 26, 1958. He was admitted to the Ophthalmological Clinic of Kyushu University on April 10, 1958.

Present status. The right eye. The outer segment normal. The fundus marked with these changes: the optic disc slightly congested and its margin ill-defined; the macular region of the retina covered with a yellowish-white and rough-surfaced exudate of a round

form, 3 P.D. or so across and some 6 D elevated above the surrounding retin a; besides a thick vitreous opacity just in front of this retinal lesion, fine opacities in the whole vitreous body, the retinal arteries normal, but the trunk of the retinal veins slightly dilatated (Fig. 1 A).

The left eye. Normal in its outer segment and fundus.

The foregoing description may largely answer to that of central exudative retinitis, but the condition identified was different from central serous chorio-retinitis common in Japan in that the retinal lesion in this case, more particularly the exudation from the superficial layer of the retina, was uncommonly severe accompanying the thick vitreous opacity in front of it. We therefore examined the whole body of the patient on the assumption that his retinitis might be ascribable to toxoplasmosis.

1. The routine examination of the body.—The x-ray of the chest showed no notable change and skull x-rays showed no intracerebral calcification. Tuberculin skin reaction weak positive. Syphilitic reactions of the blood serum and the cerebrospinal fluid negative. Electro-encephalogram normal. The differential count of white blood cells revealed slight eosinophilia (4.8%). Cerebrospinal fluid showed slight pleocytosis varying 24/3 to 50/3.

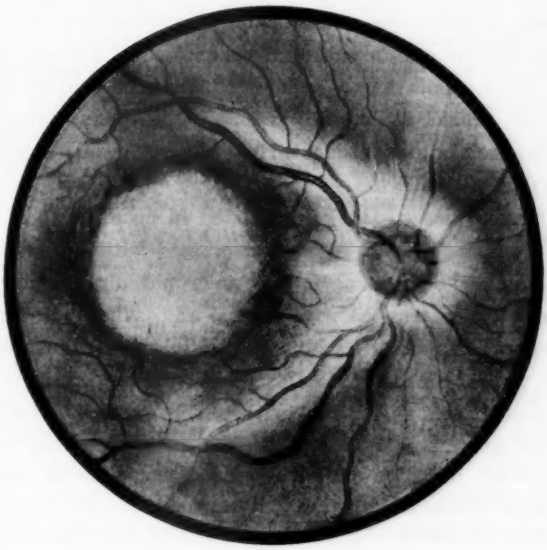
2. Special examinations for toxoplasmosis.

The dye test of Sabin and Feldman. The dye test was negative in response on April 17 and on June 29, but weakly positive, the titer standing at 1 : 4, on July 11 and on March 18 of the following year 1959.

Isolation of the parasite. The cerebrospinal fluid obtained on April 17 from the patient was intraperitoneally inoculated in a dose of 0.8 cc first in 5 mice. Serial passage was done at the interval of one week. Of 5 mice in which the 4th inoculation was made, two were found to contain the parasites in their peritoneal exudates. The smear preparation of the original cerebrospinal fluid obtained from the patient was examined and found to contain the parasites in it. The parasite isolated, a protozoan 4.5—6.2×2.0—3.5μ in size, crescent in shape, and motile, with a nucleus some 2μ across in the blunted end portion of its body, was identified as *Toxoplasma gondii* (Fig. 2).

Treatment and course. The patient, a suspected case of toxoplasmosis on April 10, was put under symptomatic treatment—salicylate and iodine therapy, and from April 22 on, predonisolone therapy in addition—till the results of different tests became known. Predonisolone was given in a daily dose of 15 mg for the first 5 days and in a daily maintenance dose of 10 mg later till the amount given totalled 150 mg. After this administration of the corticosteroid, the visual acuity of the right eye improved to 20/30, and the edema of the macular region rapidly decreased. Another 150 mg of predonisolone administred brought up the visual acuity of the right eye to 20/15 (on May 12), and dispelled the edema almost entirely. No sulfonamide was given during this treatment. Cessation of the treatment on May 22 did not result in any recidivation of the lesion. The patient was discharged on July 21. Examination on August 22

Fig. 1. Central exudative retinitis due to toxoplasmosis.
A. Before treatment.
B. After treatment.



showed that the visual acuity was 20/15 for both eyes and the macular region of the retina in his right eye was slightly pigmenied (Fig. 1B).

Comment

Toxoplasma gondii, a widely distributed parasitic protozoan in Europe and America, is now fully recognized there as a pathogen productive of a large variety of diseases, and it is said that the parasite has a high affinity for the central nervous system and the retina. An ocular lesion, as Hogan[3] and Bamatter[1] have remarked, is of special importance in the diagnosis of toxoplasmosis ; it is indeed the only symptom of the disease in no small proportion of cases.

In Japan the parasite has so far been isolated in no more than 31 cases (inclusive of the present)[10]. Four of these cases were combined with ocular lesions ; a 22-day-old female patient of memingo-encephalocele with microphthalmus and persistent pupillary membrane, a 4-year-old boy marked with mental and somatic underdevelopment and with macular degeneration of both eyes, a 30-year-old male patient with retinal detachment, and the patient descibed in this paper. These last two are the only cases of adult ocular toxoplasmosis so far met with in this country. But a recent systematic investigation directs increasing attention to the possibility that this disease, though hitherto neglected as an infection of rare occurrence, may be considerably high in incidence, occurring as a latent infection. The proportion of cases in which serum from normal Japanese is positive (1 : 16 and more) in the Sabin-Feldman dye test is estimated at 6.7% by Tsunematsu et al[7], and at 7.2% by Hasegawa et al[2]. Omura & Kashiwagi[6], ophthalmologists, who examined by the toxoplasmin skin test a number of their out-patients chosen at random, found that the response to the test was positive in 20 and dubiously positive in 21 ; the ocular lesions noted in the former group were central chorio-retinitis, macular degeneration of the retina, persistent pupillary

Fig. 2. *Toxoplasma gondi* isolated. ×1140.
Arrows indicate *Toxoplasma*

membrane, high myopia and so. Iwase[4], also an ophthalmologist, who made the toxoplasmin skin test in 97 cases, saw that a positive response was given in 24.7% (most of these being patients of uveal diseases) of cases, and she found, besides, in the dye test and the complement fixation test made in 28 cases that the titer of the dye test was 1 : 64 in a case of irido-cyclitis. Tsunematsu et al who examined diseases of the ocular fundus in 69 cases found that the titer of the dye test 1 : 4 in 16 of these cases, 1 : 16 or more in 25, and maximum titer—1 : 256—in a case of chorio-retinitis.

Recently the toxoplasmin skin test and the Sabin-Feldman dye test were done in 109 in-patients with various eye diseases in the Ophthalmological Clinic of Kyushu University and we found that the skin test was positive in 18 (16.5%) of 109, and dubiously positive in 11 (10. 1%). Of the 18 cases with positive skin test reaction, the titer of the dye test amounted to 1 : 16 or more in 3, to 1 : 4 or more in 8 and to 1 : 4 or less in 7. Of the 11 cases with dubiously positive skin test reaction, the titer of the dye test exceeded 1 : 16 in one, 1 : 4 in one and fell below 1 : 4 in 9. The incidence of positive dye test reactions (the titer 1 : 4 or more) was higher in the patients with positive skin test reactions than in those with dubiously positive skin test reactions, and positive dye test reactions were higher in incidence among the cases of retinal detachment and irido-cyclitis than among those of other eye diseases: the maximum titer of dye test reaction 1 : 256 was reached in a female patient with retinal detachment. It is to be added, however, that this experiment is not conclusive in result and is still in progress as the cases in which it has so far been made were not so large in number.

It is easily inferable from the results of the above-described variety of experiments that the *Toxoplasma*, though isolated seldom in Japan, is highly causative of toxoplasmosis, manifest or latent, and probably causative of many diseases of the eye resulting there from.

The subject of this study, an adult patient with ocular toxoplasmosis, is of special ophthalmological interest in these two respects :—

1. Acquired infection with the protozoan in adult has seldom been manifested as lesion of the eye, so that toxoplasmosis may appear to be unnecessary in the differential diagnosis of eye diseases in an adult patient. However, the present report of a case of adult toxoplasmosis, the results of our skin test and dye test, the well-known report of Wilder[8] in the Armed Forces Institute of Pathology in Washington who reexamined the slides of many eyeballs preserved there and found the protozoa in chorio-retinal foci, and the report of Jacobs et al.[5] who isolated the parasite from the intra-ccular tissue of an adult human eye suffered from chorio-retinitis—these all combine to prove that a toxoplasmic infection of an adult human eye is by no means a condition of rare occurrence.

2. The protozoan pathogen has occasionally been isolated from some tissue in post mortem examination of a person with acquired toxoplasmic infection, but very rarely

from some tissue or body fluid of an infected individual still alive (Woods[9]). In our patient who was an adult male the parasites were isolated from his cerebrospinal fluid, and his ocular lesion was remarkably amenable to treatment.

Conclusion

An adult male patient with central exudative retinitis was examined and the causal agent *Toxoplasma gondii* was found in his cerebrospinal fluid. The parasites were isolated in mice after intra-peritoneal inoculation with the cerebrospinal fluid.

(Published in J. of Clinical Ophthalmology 13: 1485, 1959 in Japanese.)

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GENETIC STUDY ON RETINITIS PIGMENTOSA

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Introduction

The author has examined in particular about a pedigree of retinitis pigmentosa, studying early changes of this disease, the mode of inheritance and the relations between this disease and others, especially between high myopia and retinitis punctata albescens.

Method of Examination

This study was made during the period of two and a half years from September 1956 to March 1959. The proband is V-2 in the attached pedigree chart. A majority of the members of this pedigree were called for medical examinations to the Ophthalmology Clinic of our Hospital, while other members including those of very old age were examined at their homes. Seventy-four members in total were examined, i.e.,

64 blood relatives and 10 spouses who had descendants (except 5 whose conditions before death were unknown, 1 who expunged his name from the family register, and 7 under two years of age). There were recorded 7 still-births. To begin with taking their medical history, the examinations were made for the visual acuity, field of vision, color vision by Ishihara's charts and direct and indirect opththalmoscopic examination for all examined individuals, and the dark adaptation test for 18 of them. Luetic reaction by Ogata was tested for 33 members, none of whom showed positive reaction. Blood types examination by using anti-A, anti-B and anti-N sera disclosed no sign of incorrect parentage. The results of the examinations are given in detail in the chart.

Result

a. In this pedigree, 60 members underwent thorough ophthalmic examinations on retinitis pigmentosa and myopia. Other two, VI-1 and V-38, were normal in their ophthalmoscopic appearance, but it is not certain whether they had refractive error or not, and additional two, III-7 and V-4, had worn glasses for short sightedness, but unknown whether they had been affected with retinitis pigmentosa or not. Consequently, information was obtained in total of 62 members either for myopia or retinitis pigmentosa, of whom 28 (12 males and 16 females) were affected with retinitis, and 34 (12 males and 22 females) were myopic.

b. Nearly all retinitis pigmentosa patients had myopia; of 28 cases of retinitis pigmentosa, only one case, IV-1, had not showed myopia, of 34 cases of myopia, five cases, V-7, 15, 21, 34, and IV-29 (all females) had not showed signs of retinitis pigmentosa at the examination.

c. Ophthalmologic examinations disclosed that male patients had typical signs of retinitis pigmentosa, whereas female ones in general atypical signs or those similar to retinitis pigmentosa sine pigmento.

d. Many infants showed very slight fundus change, which might not always be pathologic.

e. No case of congenital abnormality of color vision (red and green blindniss or incomplete color blindness) was found.

Disussion

The early changes in the fundus of retinitis pigmentosa

Though Crick, and Goodman and Gunkel has described a few findings seemingly early ophthalmoscopic change of this disease, no one has observed the whole process from the beginning stage to developed retinitis pigmentosa.

In author's pedigree, two cases, V-23 and V-30, showed a slight fundus change in the early stage, which developed into a typical retinitis pigmentosa within two years and a half. At the first examination the following appearance was found in fundus of V-30: choroidal vessels were seen more vividly than those in normal, but retinal vessels were normal; retina was coarse in entire periphery and dark brownish yellow

It seems to be improbable that such a close correlation between retinitis pigmen-

in posterior polar region, losing its normal appearance and showing abnormal reflection just like than of the metal surface. At the second examination the following were noticed: narrow retinal vessels, and narrow irregular and pale spots with some pigments in their inner part were observed here and there; fine pepper and salt fundus was seen at some places outside the above memtioned areas. On the last examination, two and a half years after the first examination, very small typical pigmentation was observed along vessels. Thus, the development of retinitis pigmentosa had been observed from its early beginning. A similar process is observed in the experimental retinitis pigmentosa.

On the retinitis pigmentosa and socalled retinitis punctata albescens

In this pedigree, there were many female patients who showed atypical ophthalmoscopic changes. In the fundus of three sisters (IV-24, 25, 27), children of a father who had a typical retinitis pigmentosa, many peculiar white spots were seen Similar change was seen in fundi of IV-8, 11, 13, 14. In their fundus, as seen in that of retinitis pigmentosa sine pigmento, a little pigmentation was observed. This inclination was recognized in the slightest degree in IV-22, whose female offspring, however, was affected with typical retinitis pigmentosa. Judging from this finding, it is apparent that white spots and dots or pigmentation appeared in the fundus may be due to the variable expressivity of a single gene. Lauber has classified retinitis punctata albescens into the following two groups, i.e., the first group of congenital stationary hemeralopia with scattered white spots and the second group of congenital progressive hemeralopia, fine pigmentary change similar to retinitis pigmentosa, choroidal atrophy, narrow vessels and optic nerve atrophy. In my opinion, the second group seems to be a type of retinitis pigmentosa, and not one group of retinitis punctata albescens.

On myopia and retinitis pigmentosa

Many of retinitis pigmentosa patients are reported to associate myopia. According to Falls 50% of retinitis pigmentosa cases accompany myopia. In this pedigree, out of 28 retinitis pigmentosa patients only one was not myopic, and out of 32 myopic patients five (all females) had not retinitis pigmentosa; that is, the percentage of association is far higher than 50% reported by Falls. These two disorders had been considered to be of different nature. However, the close correlation between retinitis pigmentosa and myopia observed in this pedigree suggests some developmental relationship between the two. Usher's and Niimi's pedigrees are extremely interesting from this point of view. In the both, retinitis pigmentosa was inherited as a sex-linked recessive trait. In Usher's 11 retinitis pigmentosa male patients excluding one case unexamined and all the carriers (female) were myopic. In Niimi's in which atypical retinitis pigmentosa was inherited through two generations, five patients and 2 carriers (female) were all myopic.

It seems to be improbable that such a close correlation between retinitis pigmen-

tosa and myopia is due to genetic linkage between two independent genes responsible for these two characters. Most probable explanation for the above mentioned findings in Usher's, Niimi's and the present author's pedigrees is that the two abnormalities are pleiotropic effects of a single gene. IV-1 in the present author's pedigree had retinitis pigmentosa without myopia; however, her father and two sons possessed both abnormalities together. Therefore, the absence of myopia in IV-1 should be accounted by incomplete penetrance rather than by genetic segregation. On the contrary female carriers in Usher's pedigree showed myopia without retinitis pigmentosa. In the present author's pedigree, five female cases, V-7, 15, 21, 34 and IV-29 had showed myopia, but no sign of retinitis pigmentosa. Of these 5 individuals four are yet unmarried and one is married but childless. It is possible that offspring to be born from these 5 myopic individuals in future may be affected with retinitis pigmentosa.

Retinitis pigmentosa inherited as a sex-linked dominant trait

As the modes of inheritance for retinitis pigmentosa six types have been reported, but no pedigree of sex-linked dominant type had so far been known. It seems to be almost doubtless on the following reasons that the abnormality in the present author's pedigree, except the uncertain point of the 2nd generation which could not be examined, is due to a sex-linked dominant gene.

First, in the present author's pedigree retinitis pigmentosa and myopia can be regarded to of dominant inheritance.

a. The disease was transmitted for three successive generation without skipping.

b. About a half of the children born of affected parents were affected with the same disease: i.e., among 43 children whose parents had retinitis pigmentosa, 23 were affected by the disease and 20 were not. This ratio is not significantly different from the expected ratio 1 : 1 in simple dominant inheritance. And among 41 children whose parents had myopia, 24 were myopic and 17 were not. This ratio is also not deviated from the expected one. Regarding the two inherited characters as one syndrom, among 47 children whose parents had the two characters or either one of them, 29 were affected and 18 were not. This ratio is also the same as the above.

c. All the parents of retinitis pigmentosa patients were affected by retinitis pigmentosa, the penetrance being 100%. As for myopia, among 12 parents of myopia patients, 10 had myopia and 2 had not, the penetrance being 83%. Among 14 parents of patients who had the one or the both, 12 had the one or the both, the penetrance being 85%.

d. In this pedigree there were 10 sibships having retinitis pigmentosa, of which 3 were consanguineous ones, while there were 3 sibships having not retinitis pigmentosa, all of which were born of not consanguineous marriage. However the retinitis pigmentosa in this pedigree may not be a result of consanguinity, though this pedigree is highly inbred.

In a word, the retinitis pigmentosa in this pedigree is, without doubt, controlled

by a dominant gene with a high penetrance.

Secondly, there is a question left as to whether the gene is located on autosomes or sex chromosomes.

e. In this pedigree, the gene is transmitted to the next generation through male line as well as female line. The penetrance through one line does not seem to be different from the degree through the other line. But, in general, female patients are more slightly affected than males in this pedigree. As to inherited characters of autosomal inheritance, there can be no difference in its genotype between males and females. But difference in expressivity according to sex is often observed. That may be the case in this pedigree, too. But, sexual difference may be caused, of course, by difference of genotype, as in the case of sex-linked inheritance.

f. According to the literature, the pedigree of the congenital eye disease which accompanies myopia is transmitted as sex-linked.

g. All 11 male offspring born of fathers having both retinitis pigmentosa and myopia were normal, while among 10 female offspring of affected fathers, 1 had myopia, 1 had retinitis pigmentosa and 8 had both. If retinitis pigmentosa is controlled by an autosomal dominant gene, probability to give such a result is 0.02% by Fisher's direct method.

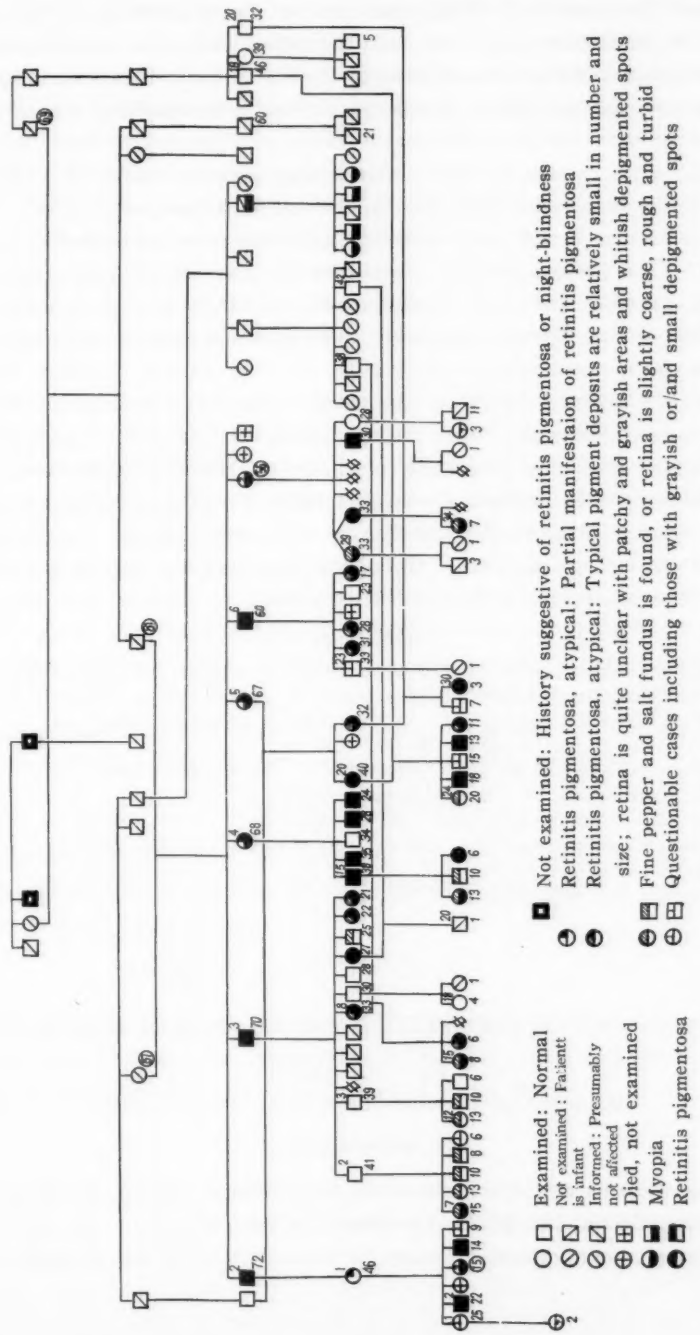
The above mentioned 11 male children had no refractive error, while among the 10 female children, 9 had myopia; the probability showing this distribution is lower than 0.02% which is extremely small. If these two characters constitute a syndrom, male children of affected fathers are all normal, and female children of the affected fathers are all affected with no exception. Probability to give such a result is quite small for autosomal inheritance. Incompletely sex-linked dominant inheritance was excluded in this pedigree, by a thorough analysis of the family data. Prof. Curt Stern insisted that this pedigree was of autosomal dominant trait or sex-linked dominant trait, even though there were some exceptions. According to his opinion, if assumed the former type of inheritance, distribution given in (g) is of very low probability, and the exception, assuming the latter, is V-21. But if retinitis pigmentosa and myopia compose a syndrom, V-21 is not an exception. If the syndrom is transmitted as a sex-linked trait, however, there is difficulty to be explained. Since I-3 and I-4 were reported to be affected, the abnormal gene seems to transmitted to III-2, 3 and 6 passing through I-2 (female) and II-5 (male). Even if I-2 (female) is assumed to be of incomplete type and II-5 (male) is not penetrant, the sex-linked gene cannot be transmitted from male (II-5) to males (III-2, 3 and 6).

Excepting this difficulty, it is most probable that retinitis pigmentosa and myopia, constituting a syndrom, are controlled by a sex-linked dominant gene.

Summary

1 In the course of study on the inheritance of retinitis pigmentosa, the author

Fig. 1. Pedigree chart of retinitis pigmentosa.


Examined: Normal
Not examined: Patient is infant
Informed: Presumably not affected
Died, not examined
Myopia
Retinitis pigmentosa
Not examined: History suggestive of retinitis pigmentosa or night-blindness
Retinitis pigmentosa, atypical: Partial manifestaion of retinitis pigmentosa
Retinitis pigmentosa, atypical: Typical pigment deposits are relatively small in number and size; retina is quite unclear with patchy and grayish areas and whitish depigmented spots
Fine pepper and salt fundus is found, or retina is slightly coarse, rough and turbid
Questionable cases including those with grayish or/and small depigmented spots

has found a pedigree in which the trait has been transmitted for three generations as a sex-linked dominant trait which has not yet been recorded in the literature. This mode of inheritance has not been described for this disease, though the existence of such a trait had not been denied. By the author's discovery of this pedigree, all theoretically possible modes of inheritance has been described for retinitis pigmentosa.

2 Slight fundus change indicating chorioretinal atrophy was found in two females of 3 years old in the pedigree, though no subjective complaint had been noticed. This slight change had developed to a typical retinitis pigmentosa when examined two and half years later. This finding indicates that retinitis pigmentosa begins with a slight chorioretinal atrophy, and such a slight fundus change will serve as a clue to the early diagnosis of the disease. Such an observation is possible only by a pedigree study as this.

3 One of the patients (IV-27) in this pedigree showed a fundus change just like retinitis punctata albescens. This finding indicates that some cases of so-called retinitis punctata albescens may be atypical form of retinitis pigmentosa.

4 The study of this pedigree shows that there is a group of retinitis pigmentosa which accompanies myopia. It was shown that the myopia accompanying retinitis pigmentosa is not a linkage of two genes but pleiotropic effects of a single gene producing fundus change as well as refractive error.

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STUDIES ON THE THEORETICAL VALUES OF LENS FLEXIBILITY

Ryoichi YAMAJI

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Introduction

The theoretical values of lens flexibility have been unknown satisfactorily. The result of calculation on this problem is shown in this paper.

The range of several elements necessary for the calculation of lens flexibility

One of combination of several elements necessary for the calculation of lens flexibility is the combination of cornea flexibility (D_1), total eye flexibility (D), the distance between the second main point of lens (d) and the refractive index of aqueous and vitreous humour (n). While the calculation can be performed using the other combination, this combination is used in this paper at my convenience.

As these elements are variable except n is constant (1.336), it is necessary before the calculation to decide the range of them.

It had been already reported by author[1] in 1952 that the range of D_1 is 40.418–48.076 D, on the assumption as the corneal first radius (r_1): 7.0–8.0 mm, the corneal second radius (r_2): 6.0–7.0 mm, the refractive index of cornea: 1.376, the refractive index of aqueous humour: 1.336 and the thickness of cornea: 0.5 mm. As the value of r_1 and r_2 measured exist almost among these values and the refractive index of cornea and aqueous humour and the thickness of cornea are about constant, this range of D_1 is supposed to be right.

The range of D is assumpted as 60.0–80.0 D. This range lean to myopia too much because the total eye flexibility of Gullstrand's model eye which has+1.0 D for the main point flexibility is 58.64 D. But, the lens flexibility is discussed usually in the problem of myopia and the value of lens flexibility under 60.0 D or over 80.0 D shall be able to be presumed easily from this results.

The range of d is seted in 4.0–8.0 mm. The range of the position of the corneal second main point had been already calculated[1], but that of the first main point of lens have been unknown. The variability of the former is about 0.02 mm under the same assumption as D_1. Then, it is enough, if the range of variable d is 4.0 mm. Rather, it is supposed that its range is too wide, but it may be corrected when the range of the first main point of lens become clearly to be known.

Method of Calculation

In this paper, the theory of the light near the axis is used as real approximate calculation. From the equation on the combination of two lenses.

$$D = D_1 + D_2 - \frac{d}{n} D_1 D_2$$

$$\therefore D = \frac{n(D - D_1)}{n - dD_1}$$

Selecting 5 values from the each range of D_1, D and d, as D_1=40.0, 42.0, 44.0, 46.0, 48.0; D=60.0, 65.0, 70.0, 75.0, 80.0; d=0.004, 0.005, 0.006, 0.007, 0.008, and using n=1.336, the calculation is performed about all combination of these values.

Results of Calculation

The values of D_2 thus calculated are shown in Table 1. From this table, it is recognized that the values of D_2 exist among 14.014 D--52.598 D within these range of elements.

Table 1.

D_1	d \ D	60.0	65.0	70.0	75.0	80.0
40.0	0.004	22.721	28.401	34.082	39.762	45.442
	0.005	23.521	29.401	35.282	41.162	47.042
	0.006	24.380	30.474	36.569	42.664	48.759
	0.007	25.303	31.629	37.955	44.280	50.606
	0.008	26.299	32.874	39.449	46.024	52.598
42.0	0.004	20.589	26.308	32.027	37.747	43.466
	0.005	21.350	27.290	33.222	39.155	45.087
	0.006	22.180	28.347	34.509	40.672	46.834
	0.007	23.079	29.489	35.900	42.311	48.722
	0.008	24.048	30.728	37.408	44.088	50.768
44.0	0.004	18.428	24.186	29.945	35.703	41.462
	0.005	19.154	25.140	31.125	37.111	43.097
	0.006	19.940	26.172	32.403	38.634	44.866
	0.007	20.794	27.292	33.790	40.288	46.786
	0.008	21.724	28.512	35.301	42.089	48.878
46.0	0.004	16.236	22.035	27.833	33.632	39.431
	0.005	16.911	22.951	28.991	35.031	41.071
	0.006	17.645	23.947	30.249	36.551	42.853
	0.007	18.446	25.034	31.621	38.209	44.797
	0.008	19.322	26.223	33.124	40.025	46.926
48.0	0.004	14.014	19.853	25.692	31.531	37.371
	0.005	14.628	20.723	26.818	32.912	39.007
	0.006	15.298	21.672	28.046	34.420	40.794
	0.007	16.032	22.712	29.382	36.072	42.752
	0.008	16.840	23.857	30.874	37.891	44.908

Graph of ΔD_2 for Δd

The variability of D_2 values for the variaty of these elements values is known from the Table 1. Next, this variety shall be shown in graph. While the graph can be gained by plotting the values of the Table 1, the graph of the curve of equation is better in continuity.

In $D_2=\frac{n\ (D-D_1)}{n-dD_1}$, let variable D_2 and d set y and x,

then, $y=\frac{n\ (D-D_1)}{n-D_1x}$

$$y'=\frac{n\ (D-D_1)}{(n-D_1x)^2}\cdot D$$

$$y''=\frac{2nD_1^2\ (D-D_1)}{(n-D_1x)^3}$$

$D>D_1$, $n-D_1x>0$

$\therefore y''>0$

then, minimum value is gained.

extremely value: $y=0$ $(y'=0\rightarrow x=-\infty)$

but, because $x>0, y>0$, the curve may be graphed in the first quadrate,

$n-D_1x>0$, $x>0$

$$\therefore \frac{n}{D_1}>x>D$$

then, $\infty>y>D-D_1$

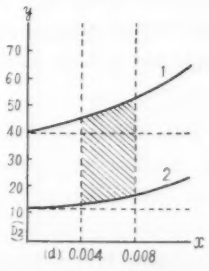
and $D_1=40-48$, $D=60-80$,

$0.0334-0.0278>d>0$

$\infty>D_2>40-12$

And so all curves in any combination exist between the curve 1 (passing on $x=0$, $y=40$, asymptote: $x=0.0278$) and the curve 2 (passing on $x=0$, $y=12.0$, asymptote: $x=0.0334$). In the area surrounding from these 2 curves and the straight lines of $x=0.004$, $x=0.008$, exist the values of Table 1 and this area is the area of D_2. (see Figure 1)

Fig. 1.



The maximum of ΔD_2 for Δd exist near of $d=0.008$ in the curve 2 and D_2 varies 1.992 D for the variation of d 0.001. The minimum of ΔD_2 for Δd exist near of $d=0.004$ in the curve 1 and D_2 varies 0.614 D for the variation of d 0.001.

Summary

As the results of calculation, it was recognized that:

1. The values of the lens flexibility were 14.014 D —59.598 D on the assumption as the cornea flexibility: 40.0 D-48.0 D, the total flexibility of the eye: 60.0 D-80.0 D, the distance between the second main point of cornea and the first main point of lens: 4.0 mm-8.0 mm and the refactive index of aqueous humour and vitreous humour: 1.336.

2. The variable quantity of lens flexibility were 0.614 D-1.992 D, when the variable quantity of the distance between the second main point of cornea and the first main point of lens was 1 mm.

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STUDIES ON ADENOVIRUS TYPE 11 INFECTION OF THE EYE

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Many reports have been made about the studies on adenovirns infections of the eye, such as the epidemic Keratoconjunctivitis (EKC) and pharyngoconjunctival fever. Adenovirus type 1 to 17 exept type 12 and 13 have been isolated from conjunctivitis. Especially type 8,7 and 3 are known as types followed by punctate keratitis and also about type 11, Dr Hirota[1] recently succeeded to isolate one strain from a patient suffering from epidemic keratoconjunctivitis and to make punctate opacities on the cornea at the human volunteer's test.

For a while, Dr. Benett et al[2] succeded to isolate one strain from the patient suffering from conjunctivitis without punctate keratitis.

Literature did not show what etiologic agent is Adenovirus type 11, and also Hirota, Benett et al, have not studied Adenovirus type 11 infection in detail.

Now, we have suceeded to isolate two strains of the Adenovirus type 11 from the conjunctival scrapings of the clinical EKC patient and performed such experiment as described in a later section for the puropse of pursuing the part which Adenovirus type 11 is playing on epidemic Keratoconjunctivitis.

Experiment

1) Virus isolation

Object: Patients who visited our Department of Ophthalmology, Branch Hospital of the University of Tokyo and were diagnosed clinically as epidemic Keratoconjunctivitis.

Methode: Conjunctival scrapings were put into 0.3 ml of YLE medium containing 10% chick serum. YLE medium was Earle's balanced salt solution containing yeast extract 0.1% and lactalbumin hydrolysate 0.5%. These were stored at—20°C in a deep freezer.

After the growth medium (YLE medium+15% cattle serum) of HeLa cell tube was washed twice with Hank's solution, being changed into maintenance medium (YLE medium+10% chick serum), with these media the material were inoculated.

After the cultivation at 36°C, the cell tube was observed every day, and the passage was made at the interval of 1~3 weeks until the cytopathogenic effect was observed.

2) The inoculation on human volunteers

Object: 17 human volunteers who have not suffered from conjunctivitis and had no changes at the conjunctiva and cornea.

Adenovirus type 11 prototype "Slobiski" strain was cultured for many times in a HeLa cell tube. After the cytopathogenic effect was completed, the mixture of the infected cell and culture fluid was repeatedly frozen and thawed for five times, and clarified by centrifugation at 3,000 rpm for 30 minutes, with resulting supernatant.

Method: One eye of each volunteer was inoculated with infected tissue culture fluid, the other eye was for control.

3) Serological investigations

Object: 116 patients of clinical EKC, who visited the Department of Ophthalmology, Branch Hospital of the University of Tokyo from 1957 to 58, were divided into three groups. A group, 1 to 15-years-old, B group 16 to 35-years-old, C group above 36-years-old. Blood was gathered of each group of patients in their acute and convalescent stage. These gathered sera were inactivated at 56°C for 30 minutes.

Neutralization test.

The neuralization test was performed in HeLa cell culture. The maintenance medium was YLE medium containing 2% horse serum. The virus was diluted in the maintenance medium so that the dilution might contain an amount of virus just enough to produce complete cytopathogenic changes in 3 days.

One volume of virus dilution and one volume of serum dilution were mixed and the mixture was held at 37°C for 30 minutes. Then 8 volumes of the mediums were added.

One ml of the mixture was then transferred to each of the two HeLa cell cultures. As a virus control, 2 tubes of cell culture inoculated with the virus dilution mixed with the medium instead of serum were included.

The test was read 48 hours after the virus controls had reached complete cytopathic changes. The criterion of neutralization was as follows: If complete prevention of the cytopathogenic effect occurred, the result was considered as definite neutralization. The titration end-point was taken as the highest dilution of the serum which gave definite neutralization. Each 0.1 ml amount of the serum dilution at the end-point was considered to contain one unit of antibody.

4) Distribution of neutralyzing antibody titer of the adenovirus type 11 in the serum of normal infants and adults.

Object: 58 samples of serum of healthy infants that visited the Department of Pediatrics of the Branch Hospital of the University of Tokyo were used.

Result

1) Virus isolation: Two-strains of "Ieki" and "Isobe" of the adenovirus type 11 were isolated from conjunctival scrapings of clinical EKC patients.

Isolation of Ieki-strain:

Conjunctival scrapings of the 2nd day of the illness were inserted into a HeLa cell tube, and observed for 8 days. The second passage was made 15 days later.

After the third passage was made, on the first day the cytopathogenic effect was noted.

This strain was identified as adenovirus type 11 by the neutraization test. The neutralizing antibody response against adenovirus type 11 was not found on the 20th day of the illness, but the four-fold rise of the neutralizing antibody titer was observed 180 days after the onset of the illness, and the neutralizing antibody response against adenovirus type 8 was not found throughout the illness.

Clinical record of the patient from whom Ieki-strain was isolated

On April 6th 1958, a foreign body feeling and hyperemie of the conjunctiva appeared on the left eye. At the first examination on May 5th, hyperemie follicle and opacity revealed itself in the conjunctiva of the left eye, and after 2 weeks acute symptoms of follicular conjunctivitis disappeared.

The two smallest epithelial opacities appeared at the cornea on the 14th day of the illness, disappearing after three days. On the right eye, a slight conjunctivitis came out after 9 days when appearing on the left eye, and disappeared without manifestation in the corneal epithelial opacities.

Isolation of Isobe-strain; Conjunctival scrapings of the third day of the illness were inserted in the HeLa cell tube, being observed for 14 days. The second passage made the cytopathogenic effect on the 21st day of the illness. As the result of the neutralizing test this strain was identified as adenovirus type 11.

Neutralizing antibody titer against the adenovirus type 11 in the serum of the patient was less than 8 on the third day of the illness and on the 33rd day of the illness, it was above 32. Neutralizing antibody titer rise against the adenovirus type 7 and type 8 was not found.

Clinical record of the patient from whom Isobe-strain was isolated

On September, 6th 1958, the patient found hyperemie of bulbar conjunctiva of the left eye but no sighn of fever, pharyngitis and diarrhoe. Although there were many follicles, heavy hyperemy, and swelling was found in the conjunctiva at the first examination on September 8, a pseudomembrane was not found. On the 23rd day of the illness an epithelial punctate opacity was seen on the under surface of the cornea of the left eye, but on the 32nd day of the illness it disappeared. Though the conjunctival symptom of the left eye lasted for 2 weeks, that of the right eye remained normal. Preauricular adenopathy was found since the first examination.

2) Observation of volunteers

Conjunctivitis developed from 2 to 7 days after inoculation in all among the 11 cases of the group belonging to the neutralizing antibody titer less than 4 before inoculation, though all of the cases having a neutralizing antibody titer above 8 remained normal. When a clinical picture of the adenovirus type 11 infection was noted, fever was found in one out of 11 cases manifesting itself within only a day.

Pharyngitis, two out of 11 cases, diarrhoea, none out of 12 cases and preauricular adenopathy, ten out of 11 cases. The slight epithelial punctate opacities developed in two out of 11 cases, and the typical subepithelial opacities of the cornea were the same in number.

3) Neutralization test against the adenovirus type 11, with paired sera from patients who were diagnosed clinically as EKC. When 116 cases of EKC patients were examined by neutralization tests against the adenovirus type 11, 4 patients revealed a four-fold or greater rise between the acute stage and the convalescent stage, and one patient among four, showed a similar titer against type 8 and 7. So 3 patients (2.8%) had a fourfold or greater rise of neutralizing antibody titer against adenovirus type 11 (Table 1).

Table 1. Neutralizing Antibody Titer against Adenovirus Type 11 of Patients who were diagnosed clinically as EKC

Group	No. Patient	Neutralizing antibody response (four-fold or greater rise)
A-Group (2-15 yr)	37	1 (2.7%)
B-Group (16-35 yr)	49	2 (4.1%)
C-Group (above 36 yr)	30	1 (3.3%)
Total	116	4 (3.4%)

Table 2. Neutralizing Antibody Titer (>8) against Adenovirus Type 11 in Nonmal Human Sera

Age	No. sera	Neutralizing antibody titer against Type 11 (>8)
A-Group (2-15 yr)	74	13 (17.6%)
B-Group (16-35 yr)	49	14 (28.6%)
C-Group (above 36 yr)	30	7 (23.3%)
Total	153	34 (22.2%)

Table 3. Neutralizing Antibody Titer (>8) against Adenovirus Type 11 in Normal Infant's Sera

Age	No. sera	Neutralizing antibody titer against Type 11 (>8)
2~ 3 yr.	9	0
4~ 5 yr.	13	3 (23.1%)
6~ 7 yr.	16	4 (25.0%)
8~10 yr.	20	3 (15.0%)

4) Distribution of the neutralizing antibody against the adenovirus type 8 in the sera of normal infants and adults

Thirty four out of 153 cases showed a neutralizing titer above 8 against the adenovirus type 11 (Table 2). And when investigations were carried out in detail with a infant group, it was known that a neutralizing antibody was possessed since t he 4th, or 5th, year of age. (Table 3).

Discussion

As for the etiologic agent of EKC, Jawetz et al[3] isolated adenovirus type 8 from a patient suffering from epidemic keratoconjunctivitis in 1955. Since then, it became probable, that the adenovirus type 8 was the etiologic agent of EKC. At our laboratory 22 strains of the adenovirus type 8, 4 strains of type 7, and 2 strains of type 11 were isolated from clinical EKC patients until now[4]. On looking through the isolation rate, it is evident, that the adenovirus type 8 was isolated much more than any other type. Simultaneously, however, type 7 and 11 were isolated from clinical EKC patients. So one wonders whether only type 8 is an etiologic agent of EKC. For a while, Dr. Tanaka[5], who succeeded to isolate type 7 (Sekiya strain) from a EKC patient expressed her view, that though the property of acute follicular conjunctivitis was found, a punctate keratitis did not developed in her inoculation-test. Dr. Hirota, too, who isolated type 11 (Fujimoto-strain) from a EKC patients, observed that in his inoculation test typical EKC was revealed in one among two examples and subepithelial punctate keratitis developed. Since the adenovirus type 11 was isolated from the stool of patient suffering from paralytic polio by Kibrick[6], the isolation from conjunctivitis was not known except those reported by Dr. Hirota and Dr. Benett et al. Recently we isolated the adenovirus type 11, Ieki-strain and Isobe-strain from scrapings of clinical EKC patients. A punctate opacity of the cornea of isolated cases was exceedingly slight and epithelial, but the differentiation between a slight case of EKC and this could not be made. Next, investigation on the pathogenicity was performed, inoculating to human volunteers with them. When the adenovirus type 11 had contact with the conjunctiva, in the cases which had less than 4 of the neutralizing antibody titers, against the adenovirus type 11, a follicular or catarrhal conjunctivitis had appeared in all 11 cases. In two among 11 cases, (18%) developed subepithelial punctate opacity of the cornea.

Another, 3 cases (2.8%) among 116 patients, who were diagnosed clinically as EKC had a four-fold or greater rise of neutralizing antibody titer against the adenovirus type 11. Therefore, it came to be known, that adenovirus type 11 infections are few in patients, who are diagnosed clinically as EKC.

In the serum of a normal person the distribution of neutralizing antibody titer (>8) against type 11 showed to be 22%.

The distribution is a little more, than in type 7, making it equal with type 8. And it belongs comparatively to the group of low rate.

Neutralizing antibody against type 11 is not possessed by 2-3 year old infants and from 4-5 year olds it rapidly increases until the same rate as in the adult. There is no report of the distribution about the neutralizing antibody against adenovirus type 11 in Japan, and even in foreign countries it is not evident. For the infants and school children who had the neutralizing antibody against type 11 the medical history was examined, and nobody could remember suffering from conjunctivitis.

The same investigation as on type 11 was done on type 8. The result was 22 per cent. These facts suggest, that type 11 was not closely connected with conjunctivitis, as type 8 was, and it can be agreed upon the fact that there are only 2.6% that suffered from type 11 among all patients who were diagnosed clinically as EKC. Why are there a little outbreak of conjunctivitis in natural infection by type 11. It may be thought that the conjunctival epithelial cell is not the first selected place, and this fact is assumed by reason that the isolation rate of type 11 from patients who were diagnosed clinically as EKC is much smaller than that of the patients in possession of the neutralizing antibody against type 11. However, according to our human volunteer's test, when the conjunctiva was infected with type 11 virus in a very large amount, acute follicular or catarrhal conjunctivitis developes and also subepithelial punctate keratitis is possible to occur in 18% simultaneously.

These considerations suggest that the majority of the type 11 infections shows only simple slight conjunctivitis, which can not be diagnosed as a clinical EKC or slight respiratory or digestive desease hardly noticed. Furthermore, Sugiura et al[7] of our laboratory performed neutralization tests against type 3 and 7 about the same object of EKC patients. There is no patient who was found with the four-fold or greater rise against type 3 in the paired sera and there is only 3% against type 7.

From these results it is assumed, that about 90% of the patients who were diagnosed clinically as EKC in our investigation period was type 8 infection, 3% was type 7 infection, 3% was type 11, and about 3% was infection of other type of virus.

Summary.

Two strains of the adenovirus type 11 were isolated from conjunctival scrapings of the patients who were diagnosed clinically as epidemic keratoconjunctivitis. Investigation on the pathogenicity was performed in human volunteers. When adenovirus type 11 had contact with the conjunctiva, in the cases which had less than 4 of the neutralizing antibody titer against the adenovirus type 11, the following appeared: All 11 cases had acute follicular or catarrhal conjunctivitis. 2 cases among 11 cases (18%) had subepithelial punctate opacity of the cornea. Paird sera were obtained from

116 patients, who were diagnosed clinically as EKC. Among them, 3 cases (2.8%) had a four-fold or greater rise of neutralizing antibody titer against the adenovirus type 11.

In the serum of a normal person, the distribution of neutralizing antibody against type 11 began in 4-5 years aged infants. In every generation, the postive rate of neutralizing antibody titer (>8) showed to be 22%.

From the above, it was noticed, that the infection by adenovirus type 11 sometimes showed similar clinical pictures to EKC, however, the frequency was far lower and the grade of the clinical picture was lighter compared with that of the adenovirus type 8.

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EXPERIMENTAL PATHOLOGICAL STUDIES ON THE DIFFERENCE AND SIMILARITY BETWEEN TRACHOMA GRANULES AND NONTRACHOMATOUS FOLLICLES

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Concerning identity or non-identity of trachoma granules and folliculosis conjunctivae, disputes have been repeated for a long time between monistic and dualistic views. And as the consequence, has become predominant the dualistic view that the essential nature of trachoma granules, different from that of conjunctival follicles, is diffuse proliferative inflammation of conjunctiva proper, and this problem seems to have been settled almost definitely. But nevertheless disputes over it have not yet come to an end, and still various arguments are advanced over and over again.

As a means to contribute to the final settlement of this problem, the following experiments were performed by the author.

Materials and Methods

1) One per cent terramycin ointment was applied for one month to 5 cases of folliculosis conjunctivae without any inflammatory symptom. After confirming no change in the follicles, all of those found in the lower fornix of each unilateral eye were marked by tattooing under a slit lamp microscope, and then conjunctival smear from a patient with typical chronic trachoma was inoculated. At 1～2 months after the onset of trachoma, when the symptom turned into chronic phase, a part of conjunctiva containing both the tattooed follicles and newly formed granules was removed for histological observation. Immediately thereafter, the application of 1% terramycin ointment 4 times a day was started, and after 1～2 months, when the inflammatory symptom of the conjunctiva completely disappeared, and the patients seemed to have almost recovered from trachoma clinically, a section of the conjunctiva was again examined under microscope for the pre-existing follicles and the newly formed granules.

2) Apart from this, tissue specimens of granules and follicles were prepared from 35 cases—5 with acute trachoma, 5 with trachoma I, 13 with trachoma II, 5 with trachoma III, and 5 with folliculosis conjunctivae. Similar tissue specimens were also prepared from 4 cases of chronic tonsillitis, and from 3 cases of intraperitoneal lymph node. And the variety and the number of cells in the germinal center were determined.

Numerous reports have been published concerning the comparison of cells between the granules and the follicles. But all of them were made on smear specimens, and systematic investigations in tissue specimens are not yet encountered.

Results and Discussion

1) Histological findings in folliculosis conjunctivae produced by trachoma inoculation

At 1~2 months after the onset of trachoma due to the inoculation, that is before the commencement of the therapy for it, the pre-existing follicles were remarkably enlarged, and scarcely any difference was observed between these and the newly formed granules. But when the trachoma was almost completely cured, the newly formed granules largely disappeared in the majority of specimens, and the remaining ones were markedly atrophied to be observed only as small groups of lymphocytes. In contrast, the pre-existing follicles never receded despite the complete recovery from the trachoma and disappearance of their reactive enlargement and cellular infiltration due to trachomatous inflammation, presenting a peripheral zone of lymphocytes and a germinal center replete with reticular cells, lymphocytes and young lymphocytes.

In this way, in response to the onset of trachoma, the preexisting follicles underwent remarkable enlargement and proliferation, while on the other hand, numerous granules were newly formed in association with the development of trachoma. And in the chronic phase, scarcely any difference could be discerned between them. Later, however, with the advance of the treatment, the newly formed granules gradually disappeared, whereas the pre-existing follicles, having returned to the pre-inoculation state, persisted without any sigh of disappearance.

2) Comparison of cells between the follicles and granules

At various stages, trachoma granules and germinal centers of folliculosis conjundctivae were investigated for variety of cells, and their average counts were obtained. The results with the confidence and the rejection limit are represented in Figs. 1~6.

Lymphocytes. In the germinal center of folliculosis conjunctivae, lymphocytes were relatively numerous, but with advance into trachoma II and III, they gradually reduced in number. According to Takahashi, lymphocytes are numerous in granules in trachoma I, but gradually decreased with transition into trachoma II. Thus the results in the present experiments were nearly in agreement with those of Takahashi.

Young lymphocytes. There was no remarkable difference in young lymphocytes between trachoma granules at any stage and germinal centers of folliculosis conjunctivae.

Reticular cells. Reticular cells are called "Germinal center cells", since they are predominant in the germinal center of the granule. They were remarkably decreased in number in trachoma II and III in the present experiments, assumedly because they were largely transformed into degenerative cells.

Degenerative cells. According to Thygeson and Sezer, degenerative cells consititute characteristic feature of trachoma, and their appearance can be attributed to necrotic action of trachoma. Matsuo stated that the presence of degenerative cells in the

Fig. 1. Lymphocytes

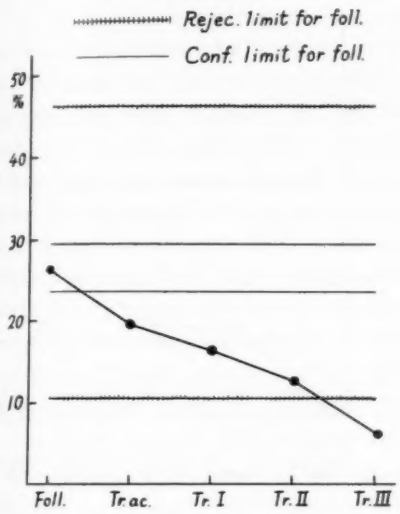


Fig. 2. Young lymphocytes.

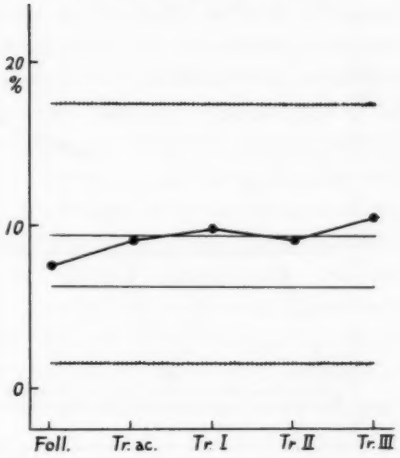


Fig. 3. Reticular cells.

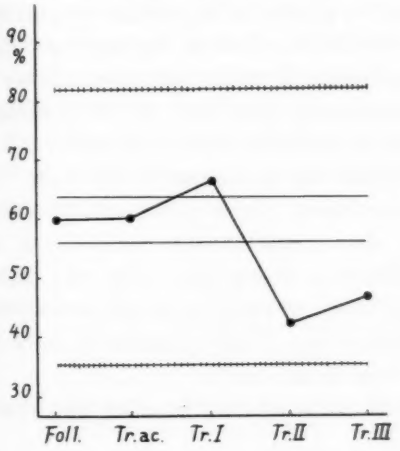
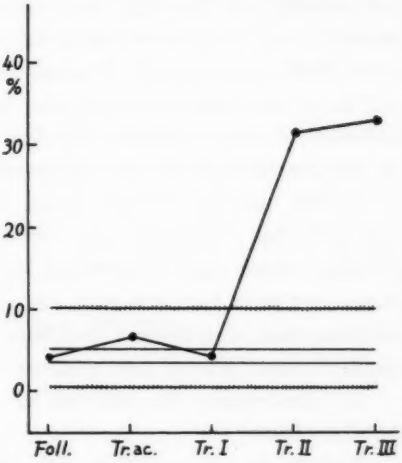


Fig. 4. Degenerated cells.



impression preparation can be used for the diagnosis of trachoma. In the present cases, degenerative cells in the germinal centers remarkably increased in trachoma II and III in agreement with the report by Thygeson and Sezer.

Macrophagocytes. Macrophagocytes, which are called Leber's corpuscles, were shown in a small number in the present cases of folliculosis conjunctivae and trachoma I, but were numerous in trachoma acute and trachoma II and III. It is therefore considered that macrophagocytes increase in trachoma granules in association with

Fig. 5. Macrophagocytes.

Fig. 6. Mitotic cells.

rise in the degree of inflammation.

Mitotic cells. Sezer and Koide reported that relatively many mitotic cells are seen in trachoma, but that they are remarkably small in number in folliculosis conjunctivae. Matsuo, Aoki, Yoshitomi and Itagaki could not consider them as the important diagnostic basis for trachoma because they are also found in folliculosis conjunctivae as well as in follicular conjunctivitis. In the present cases, they were few in folliculosis conjunctivae and trachoma III, but numerous in trachoma acute, trachoma I and II. As the result, they can be considered to be parallel with acuteness of trachoma.

In the sections of the tonsils and intraperitoneal lymph nodes, which were investigated for reference sake, reticular cells were predominantly numerous in the germinal center, and lymphocytes, young lymphocytes, degenerative cells and macrophagocytes were also observed. In other words, they presented a picture intermediate between those of trachoma and folliculosis conjunctivae. This is considered to be the result of some chronic inflammation affecting these lymph nodes.

3) Concerning the identity or non-identity of trachoma granules and conjunctival follicles

This is an old question over which disputes have been repeated by numerous workers.

Raehlman, Wolfring, Blumberg, Fujiwara, Hiwatari, Ishihara, Kunitomo and others advanced the view that trachoma granules and conjunctival follicles are identical, that difference between them is only due to difference in the degree of the same disease, and that conjunctival follicles represent incomplete form of trachoma granules. Against this, Junius, Saemisch, Peters, Greeff, Schieck, Taboriski, Pick, Lohlein, Suganuma, Wakisaka, Niimi, Aoki and others argued that

the two are completely different from each other.

In the present experiments, eyes with folliculosis conjunctivae without inflammation were inoculated with trachoma, and it was found that the pre-existing follicles were enlarged and proliferated in response to this, while on the other hand, numerous new granules were formed. In the transitional stage into the chronic phase, no distinction can be made either clinically or histologically between the pre-existing follicles and the newly formed granules. When, however, antibiotic therapy was started in this stage, the preexisting follicles, after restoration to the pre-inoculation state, remained undiminished, whereas the newly formed granules were gradually resorbed until at last they disappeared completely. It was unmistakable that the newly formed granules were attributable to the inoculation of trachoma, and they were completely removed by the antibiotic therapy. In contrast to this, the pre-existing follicles did not disappear at all, although they were restored to the pre-inoculation state by the antibiotic therapy. On account of this, the author can not support the view that the both are produced by trachoma.

It goes without saying that the essential nature of trachoma is diffuse proliferative inflammation of conjunctiva proper with multiple development of granules. Both trachoma granules and conjunctival follicles are, like tonsil and intraperitoneal lymph node, lymph follicles, which are produced as the result of response to various stresses. However, trachoma granules are specific only to trachomatous inflammation, whereas folliculosis conjunctivae is entirely independent of trachoma. In this way, there is an absolute difference between their natures.

Furthermore, the above described cytological difference between the granules and foollicles is also considered to be ascribed to a special factor, that is, trachomatous inflammation.

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HISTOLOGICAL STUDIES ON THE OCULAR TOXICITY OF α-CHYMOTRYPSIN

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Introduction

Since Barraquer reported in 1958 on his success in causing dislocation of the lens by introduction of the proteolytic enzyme α-chymotrypsin (α-ch hereunder) into the vitreous body of a patient of advanced old vitreous hemorrhage and thus being informed on the action of the drug in dissolving the zonule, in applying it for the purpose of zonulysis at intracapsular extraction of cataract with excellent results, this agent has been subjected to follow-up studies by Inoue[1], Sato[2], Tanaka[3] and A. Benedict Rizzuti[4] and its effect has been studied in comparison with that of mechanical zonulotomy. All these studies resulted in establishing the merits of this drug which is reported as free of any dangerous effect on the other tissues in the eye. Barraquer conducted fundamental experiments with this drug with rabbits' eyes, and human enucleated and blind eyes as materials, and reports that a 1 : 5.000 solution of α-ch acts selectively on the zonule without causing any perceptible change in the other ocular tissues. The effects of the drug on the ocular tissues, however, have not yet been adeqately studied from the angle of histology. Such a deficiency in histological studies on the subject induced the present author to undertake the experiments reported below for obtaining informations on the injurious effect, if any, of α-ch on the ocular tissues.

Experiment 1. Ocular Disturbances Following Intravitreous Injection of α-Ch.

Methods

One tenth ml each of physiological saline containing 0.002, 0.02, 0.2 and 2 mg of α-ch was injected into the central part of the vitreous body through the upper equator of normal white rabbits more than 2 kg in body weight; the animals were sacrificed by emboly of air through the auricular vein on the 2nd or the 14th day after the injection, their eye-balls were immediately enucleated, fixed in Zenker's solution, embedded in paraffin and celloidin, stained by Van Gieson's, Mallory's or Van Gieson's elastica method or by myelin-sheath staining (K-B staining) and histologically examined, Mochida Pharmacy's Zonulysin was used as the test agent.

Results

Changes observed on the 2nd day after injection of 0.002 mg. The bulbar conjunctiva and the extraocular muscles were found unaffected, a slight infiltration of small round cells was observed in the corneal limbus but the cornea was found normal. A small amount of exudate containing nearly no cells was found in the anterior chamber but

no cell accumulation was found in the angle. The iris was little affected. In the ciliary processes, edema, swelling and degenerative deciduation of epithelial cells were comparatively marked and slight bleeding was also observed. The choroid was not perceptibly affected. The retina was also but little affected, except in the horizontal expansions of medullated nerve fibres where the superficial blood vessels were found dilated and surrounded by infiltrations of neutrophil and small found cells, the nerve fibre layer was coarsened and a very slight collapse of the layer of rods and cones and the outer nuclear layer was observable.

Changes observed on the 14th day after injection of 0.002 mg. The part of the retina between the upper equator and the horizontal expansions of medullated nerve fibres was locally atrophied, showing rather prominent degeneration and cellapse of the layer of rods and cones and decrease of the nuclei of the inner and the outer nuclear layers, the ganglion cells being also considerably thinned down. The other tissue were little affected.

Changes observed on the 2nd day after injection of 0.02 mg. In the corneal limbus, the blood vessels were rather markedly dilated and moderately severe infiltration of small round cells was observed, in which neutrophil cells were not numerous but plasma cells were abundant (Fig. 1). A moderate amount of exudate was found in the anterior chamber, but few cells were found therein. A localized but conspicuous accumulation of neutrophil cells was found in the angle, near which cord-like anterior synechiae was observed. The cornea was little affected, except some keratic precipitates composed of neutrophil cells and deciduated cells in the main. Hypermeia and edema were mild in the iris on the anterior surface on which were found deciduated cells in a one rowed arrangement. The upper ciliary processes were severely affected, exudation being prominent, swelling being badly advanced and degenerative deciduation of the epithelial cells being also marked (Fig. 2), but in the lower ciliary processes exudation and swelling were relatively mild and the degenerative deciduation of epithelial cells was also light, though hyperemia was somewhat notable (Fig. 3). Cell components were increased in the choroid but no marked change was observed here. Changes in the retina were severe in the upper and mild in the lower half, as in the ciliary processes; in the latter, there was little change in the internal limiting membranes, nerve-fibre layers and the ganglion cells, except that the optic cells, especially, the outer segment, were found collapsed and their structure nearly obliterated, that very slight vacuolation of the inner nuclear layer and picnosis and collapse of nuclei in the outer nuclear layer were barely observable, that the pigment epithelial cells were somewhat loosened in arrangement and that a few cells were found in the vitreous body (Fig. 4). In the upper half of the retina, however, the collapse of the layer of rods and cones was prominently advanced, the ganglion cells were nearly annihilated, the nuclei of the outer nuclear layer were severely reduced in number, and mesh-works of pigments seemingly produced by the collapse of these nuclei were found reaching to the internal limiting membrane in courses similar to those of Müller's fibres; the nuclei were

evidently decreased in the inner nuclear layer but not so badly as in the outer nuclear layer, while the retina was scarcely infiltrated by cells (Fig. 5); the papilla was found hyperemied.

Fig. 1. On the 2nd day after intravitreous injection of 0.02 αmg of -ch: The corneal limbus. Rather marked dilation of the blood vessels and moderate infiltration of small round cells observable.

Fig. 2. Ditto: The upper ciliary process. Exudation, swelling and degenerative desquamation of epithelial cells apparent.

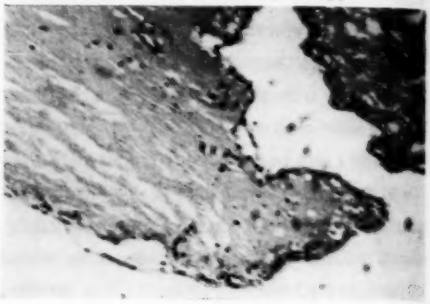


Fig. 3. Ditto: The lower ciliary process.

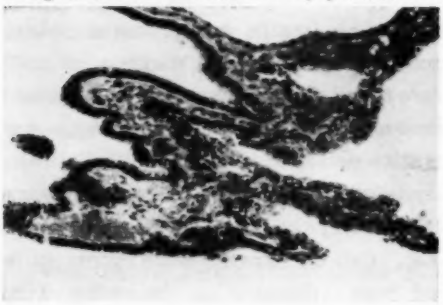
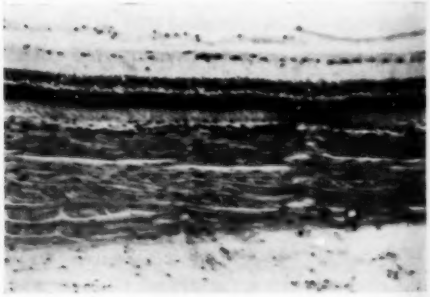


Fig. 4. Ditto: Lower part of the retina.



Changes observed on the 14th day after injection of 0.02 mg. The conjunctiva and the cornea were nearly normal, only a very little exudate and a few cells were found in the anterior chamber and no cell accumulation was seen in its angle. The iris was little affected. The exudation and edema in the ciliary processes were found markedly alleviated and regeneration of the epithelial cells, proliferation of the connective tissue and sporadic existence of siderofer cells were observable (Fig. 6). A slight infiltration of plasma cells were observed in pars plana of the ciliary body. In the upper half of the retina, adhesion to the choroid was evident, the layer of rods and cones, the outer nuclear layer and the ganglion cells were almost totally extinguished and the part consisted of little else than Müller's fibres and glia cells (Fig. 7). The pigment epithelial cells were sometimes desquamated but not subject

Fig. 5. Ditto: Part of the retina near the upper equator.

Fig. 6. On the 14th day after injection of 0.02 mg: The ciliary process. Siderofer cells in sporadic existence.

to proliferative change. The choroid in this part was affected by proliferation of fibrous tissue and plasma cell infiltration, but retrobulbar loose connective tissue was not perceptibly affected. In the lower half of the eye-ball, little change was observed in the retina and the choroid in the peripheral portion, but the collapse of the layer of rods and cones and the outer nuclear layer becomes the more remarkable as the posterior pole is approached (Fig. 8). Near the horizontal expansions of medullated nerve fibres, the layer of rods and cones and the outer nuclear layer were totally annihilated and the inner nuclear layer was in direct contact with the pigment epithelium. Here, the choroid was strongly hyperemied and hemorrhage was evident on the surface of the horizontal expansions of medullated nerve fibres.

Fig. 8. Ditto: Retina near the lower equator. The outer nuclear layer on the verge of total extinction. The inner nuclear layer only mildly affected.

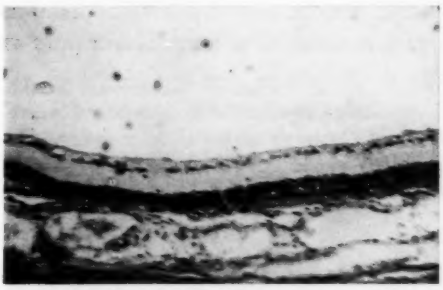
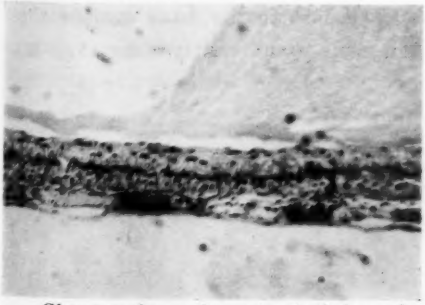


Fig. 7. Ditto: Upper part of the retina. Adhesion to the choroid observed.



Changes observed on the 2nd day after injection of 0.2 mg. The dilatation of blood vessels and small round cell infiltration were already notable in the corneal limbus but the cornea itself was little affected. Dilatation of the blood vessels and increase of cell components were observable in the bulbar conjunctiva and the stroma of the extraocular muscles. The anterior chamber was found full of exudate, but few cells were contained therein, only a small number of deciduated cells being found arranged

Fig. 9. On the 2nd day after injection of 0.2 mg: Prominent hemorrhage in the ciliary process.

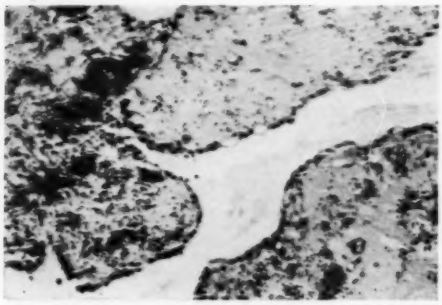
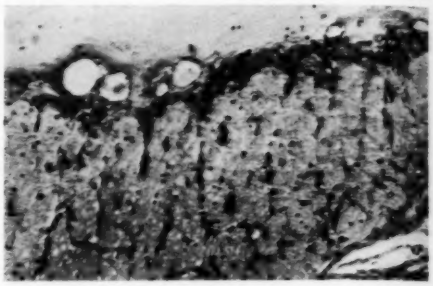


Fig. 10. Ditto: Hemorrhage in the papilla.



in a row before the iris. An accumulation of neutrophil cells similar in extent to that observed on the 2nd day after injection of 0.02 mg was found in the angle of one side. The ciliary processes were found markedly edematous and swollen, and degenerative desquamation of epithelial cells and hemorrhage were also very perceptible here (Fig. 9). The changes in the iris were milder than those in the ciliary process, consisting mainly in mild hemorrhage and desquamation of the pigment epithelial cells. A rather large number of leucocytes, erythrocytes and deciduated cells were found in the posterior chamber and the vitreous body. The choroid was hyperemied and cell-infiltrated but rather mildly. Dilatation of the central artery of the optic nerve was prominent and hemorrhage was observed around the artery, on the surface of the papilla and even within it (Figs. 10 and 11). In the retina, changes were most prominent in the upper side of the vicinity of the papilla; here, the layer of rods and cones and the outer nuclear layer were nearly obliterated and pigments were found to be arranged mesh-wise; nuclei in the inner nuclear layer were reduced to a single layer and the ganglion cells were nearly gone (Fig. 12).

Fig. 11. Ditto: Prominent hemorrhage in the horizontal expansions of medullated nerve fibres (Malory's staining).

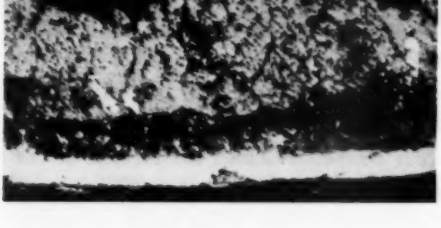
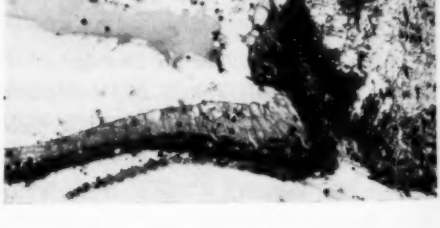


Fig. 12. Ditto: Retina near the upper side of the papilla.



The pigment epithelial cells were little affected. Near the lower side of the papilla, the internal limiting membrane was severely affected, cells closely resembling the ganglion cells being arranged in a single layer on the inner surface of the retina; the nuclei in the inner nuclear layer were markedly diversified in size and stainability, their arrangement was disordered and vacuolation was in evidence; in the outer nuclear layer, karyolysis and karyorrhexis were evident, the nuclei severely reduced in number and disordered in arrangement; the layer of rods and cones was degenerated and annulled, and mesh-like deposits of pigment were prominent in this part; the Muller's fibres were thickened and were frequently in direct touch with the pigment epithelium (Fig. 13); changes in the vicinity of the ora-serrata of both sides were only mild.

Fig. 13. Ditto Retina near the lower side of the papilla.

Fig. 14. On the 14th day after injection of 0.2 mg: Proliferation of connective tissue in the ciliary process (Malory's staining).

Changes observed on the 14th day after injection of 0.2 mg. The bulbar conjunctiva and the extraocular muscle were normalised, the hyperemia and the cell infiltration in the limbus were nearly healed and the cornea was normal. A little exudate remained in the anterior chamber but no cell was contained in it. A cord-like anterior synechiae was found near one of the angles. The cell accumulation in the angle, the hemorrhage and the swelling of the ciliary bodies were faded out, siderofer cells were found sporadically and epithelial cells were also regenerated in most parts. The proliferation of the connective tissue was comparatively prominent in the iris and the ciliary body (Fig. 14). In the retina, the part near the lower ora-serrata was nearly normal, and a small number of siderofer cells were found in the vitreous body. Little change was observable in the choroid or the pigment epithelial cells in this part. But as the posterior pole was approached, the collapse of the visual cells and the decrease of the nuclei of the outer nuclear layer cells became more prominent and the pigment epithelial cells became more rounded. The inner nuclear layer, however, was less affected. In the upper half of the retina, the changes were more drastic, the layer of rods and cones, the nuclei of the outer nuclear layer and the ganglion cells being nearly all extinct (Fig. 15 and 16). The blood bled on

the surface of the papilla had been resorbed and a number of fibroblasts and small round cells were found here (Fig. 17).

Fig. 15. Ditto: Retina near the upper equator.

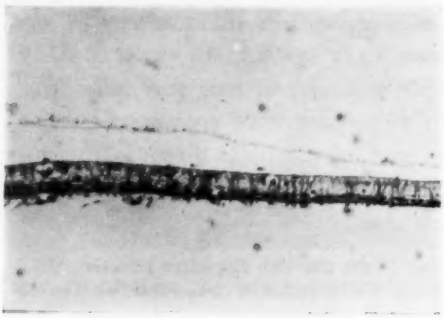
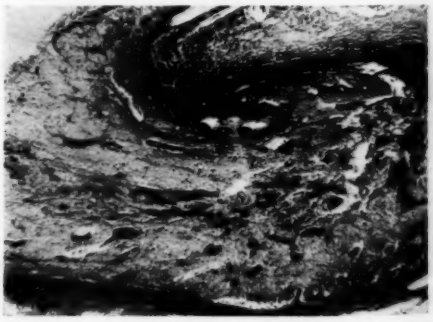


Fig. 16. Ditto: Retina near the upper side of the papilla.

Changes observed on the 2nd day after injection of 2 mg. Increase of cell components in the stroma of the extraocular muscles, edema of the bulbar conjunctiva and hyperemia and infiltration of small round cells in the corneal limbus were in evidence, but not so prominently as on the 2nd day after 0.2 mg. Plasma cells were comparatively numerous. The cornea was little affected, as in the preceding cases. The anterior chamber contained a moderate quantity of exudate but few cells. Neutrophil cells were accumulated to a moderate extent in the angle. Hemorrhage, edema

Fig. 17. Ditto: Fibroblasts and small round cells in the papilla.

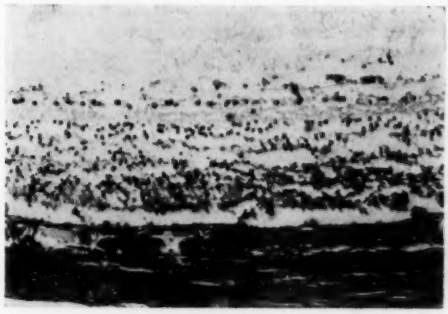
Fig. 18. On the 2nd day after injection of 2 mg: Necrosis in the optic nerve and hyperemia, neutrophil cell infiltration and hemorrhage in the optic nerve sheath.



and degenerative desquamation of the epithelial cells were observed in the ciliary process, and rather mild edema and hemorrhage in the iris. In the vitreous body, a relatively mild hemorrhage was found in the part near the retina. The choroid was hyperemied but the infiltration of small round cells was rather mild here. Prominent hyperemia, netrophil cell infiltration and hemorrhage were observable in the optic

Fig. 19. Ditto: Cell infiltration in the retrobulbar loose connective tissue.

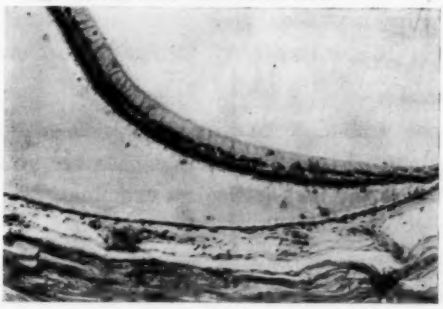
Fig. 20. Ditto: Retina near the posterior pole of the lower half.



nerve sheath, the cell infiltration extending inwards into the optic nerve (Fig. 18). Cell infiltration was considerably advanced in the retrobulbar loose connective tissue, too (Fig. 19). In the retina, changes were comparatively mild in the ora-serrata but nearer the posterior pole, degeneration and collapse of the cellular components became more evident, the lower side of the papilla being swollen and badly destroyed (Fig. 20). Near the upper side of the papilla, the thickness of the retina retained the normal thickness, but the disappearance of the cellular components was conspicuous, little else than Müller's fibres and nerve fibres being found remaining (Fig. 21). Only a few pigment epithelial cells were found on the lamina vitrea but these were intact. The optic nerve was necrotic as far as into the retrobulbar part (Fig. 18).

Fig. 21. Ditto: Retina near the upper side of the papilla.

Fig. 22. On the 14th day after injection of 2 mg: Retina near the ora-serrata.



Changes observed on the 14th day after injection of 2 mg. The bulbar conjunctiva was mildly hemorrhagic and edematous, and cell proliferation was observable in the stroma of the extraocular muscles. Dilatation of the blood vessels in the limbus was prominent and comparatively marked infiltration of small round cells were found perivascularly localised, but the cornea itself was little affected. The anterior chamber

Fig. 23. Ditto: The retina. The layer of rods and cones, the nuclei of the outer nuclear layer and the ganglion cells nearly extinct and glia cells frankly proliferated.

Fig. 24. Ditto: Prominent proliferation of the pigment epithelial cells.

was filled up with exudate, but scarcely any cell could be found in it. A small number of small round cells were found in the angle. The iris was hyperemied and the plasma cells infiltration and connective tissue proliferation were comparatively maked in it. Moderate hemorrhage was still seen in the ciliary processes and sporadic siderofer cells were found in it. The lens was dislocated, the posterior capsule was riven and the cortex in this part was found dissolved. The choroid was not much affected, except by mild hyperemia and proliferation of cell components. The retina was detached from the ora-serrata on both sides and severely destroyed (Fig. 22). The layer of rods and cones, the outer nuclear layer and the ganglion cells were all nearly gone, glia cells were prominently proliferated and nothing but these and Müller's fibres were found remaining (Fig. 23). The pigment epithelial cells were swollen and desquamated, accumulation of hemosiderine in the cytoplasm was notable and on rare occasions giant cells were in formation (Fig. 24).

Experiments 2. Ocular Disturbances Following Intracameral Injection of α-Ch

Methods

One-tenth cc each of physiological saline containing 0.02 and 0.2 mg of α-ch was injected into the anterior chamber, the eye-ball was then enucleated on the 2nd and 14th day for histological examination.

Results

Changes observed on the 2nd day after injection of 0.02 mg. The bulbar conjuctiva and the cornea were little affected and the anterior chamber contained neither exudate nor cells. In the iris a mild hyperemia and extravasation of a small number of erythrocytes were observed. Rather mild hyperemia and vacuolation in the epithelial cells were the only changes in the ciliary body. The choroid was little affected. The retina was somewhat coarsened in texture, some vacuolation and decrease of nuclei of the inner and the outer nuclear layers were barely perceptible, but otherwise no change was noticeable.

Changes observed on the 14th day after injection of 0.02 mg. The anterior chamber was nearly free of exudate and cells, only a little exudate being found on the surface of the iris. Slight infiltration of small round cells was evident in the limbus, but no other tissue was found changed to any perceptible degree.

Changes observed on the 14th day after injection of 0.2 mg.

Some proliferation of the connective tissue was observed in the iris, but the other tissues were scarcely, if ever, affected.

Summary and Discussion

In summary of the above findings on the ocular disturbances after injection of α-ch into the vitreous, we may state as follows ;

In the bulbar conjunctiva and the corneal limbus, hyperemia and infiltration of small round cells are observed on the 2nd day after the injection but the severity of the change depends little on the dosis injected and on the 14th day these tissues were found nearly normalised. No maked change was noticed in the cornea, except little deposits on the posterior surface. In the anterior chamber only some exudate and a few cells were noticed and an accumulation of some neutrophil cells in the angle, but these were found resorbed by the 14th day except after injection of 2 mg, leaving only a slight cord-like anterior synechia in the angle in the other cases. The iris was found subject to comparatively mild hyperemia, edema, hemorrhage and proliferation and desquamation of the pigment epithelial cells, but in degrees far milder than in the ciliary process, where the exudation, the swelling, the hemorrhage and the degeneration and desquamation of the epithelial cells were rather conspicuous, especially, the hemorrhage, but by the 14th day, the exudation, the swelling and the hemorrhage were alleviated and regeneration of the epithelial cells, sporadic existence of siderofer cells and proliferation of the connective tissue were apparent. In the choroid, some hyperemia and comparatively mild cell infiltration were in evidence, but these changes were far milder than similar disturbances in the retina. The optic nerve sheath was noticeably hyperemied, infiltrated by neutrophil cells and hemorrhagic in the cases injected with 2 mg of the drug, the cell infiltration extending into the optic nerves and into the retrobulbar loose connective tissue. The optic nerves were merely hyperemied after 0.02 mg, also hemorrhagic after 0.2 mg but fallen into necrosis as far as in the retrobulbar portion after 2 mg.

On the other hand, the changes in the retina were far more serious and permanent, except in the cases treated with the smallest dosis of 0.002 mg. After 0.02 mg, by the second day the lower half of the retina was scarcely affected except some degeneration of the layer of rods and cones, but in the upper half the ganglion cells were nearly gone, the outer nuclear layer nuclei were extremely reduced in number and pigment particles probably produced by collapse of these nuclei were found aligned in mesh-like courses similar to the net-works of Müller's fibres and reaching as far as the

internal limiting membrane. The inner nuclear layer was found remaining nearly normal but in some areas the layer was more severely affected, little alse than nuclei of Muller's fibres being found remaining. The layer of rods and cones was notably destroyed. The pigment epithelial cells were sparsely scattered on the lamina vitrea. No marked cell infiltration was found in the retina. On the 14th day after 0.02 mg of the drug, in the upper half of the eye-ball, the retina adhered to the choroid evidently, the layer of rods and cones, the outer nuclear layer and the ganglion cells being nearly extinguished, only Müller's fibres and glia cells were found remaining and even the mesh-work of pigment particles have faded out. The injury in the horizontal expansions of medullated nerve fibres is still severe but in the portion below it was distinctly better preserved, no perceptible change being observed between the equator and the ora-serrata. Thus the damages after intravitreous injection of 0.02 mg of α-ch were severer in the part of the retina above the papilla, than below it. This tendency was apparent also in the other cases whther the dosis was 0.002 mg or 0.2 mg, and was observed in the ciliary process and the choroid as well. After injection of antibiotics, however, the lesions were found severer in the lower half of the retina, as reported previously[5]. This difference is probably caused by the difference in the specific gravity of α-ch and the antibiotics.

On the 2nd day after injection in the vitreous body of 0.2 mg of α-ch, the lesions in the retina were even severer. The most severely devastated portion of the retina near the upper side of the papilla as stated above could be only slightly more damaged, but here the changes affected also the lower half, especially the area near the posterior pole to a notable extent. In this part, the internal limiting membrane was ruptured here and there, many cells, probably deciduated ganglion cells, were ranged in a row inside the retina, in the inner nuclear layer, the size and the stainability of the nuclei were markedly diversified, their arrangement was disordered and vacuolation was apparent, while in the outer nuclear layer, the nuclei were largely collapsed or disintegrated and severely reduced in number, their arrangement was disordered; the layer of rods and cones was degenerated and annihilated, the mesh-work of pigment particles was here particularly apparent in this area and the thickened Müller's fibres were found in direct contact with the pigment epithelial cells at many places. Such deposits of pigment was observable on the 2nd day in all the cases after injection of 0.02 mg or more of α-ch but was found always faded out by the 14th day. Such a finding, never obtained after using antibiotics as reported previously, is perhaps due to the effect of α-ch in speedily disintegrating the nuclear substance. After injection of 2 mg, the lesions become still severer all over, the difference in degree between the upper and the lower half of the retina becoming less marked. On the 2nd day, the retina near the lower side of the papilla becomes loosened in texture due to bad edema, the collapse of the layer of rods and cones and the decrease of nuclei in the outer nuclear layer were very perceptible, and the lesions here were

rather severer than in the vicinity of the upper side of the papilla. This is perhaps due to the fact, that the specific gravity of a solution of 2 mg in 0.1 cc of physiological saline is considerably larger than the vitreous humor. By the 14th day after the injection, the retina had been detached from the ora-serrata, badly damaged and atrophied, and the pigment epithelial cells were also subject to prominent degeneration, deciduation and proliferative change.

As detailed in the preceding, intravitreous injection of α-ch even in the small dosis of 0.02 mg was found to cause some injuries in all the ocular tissues and particularly serious ones in the retina. The damages to the bulbar conjunctiva, the corneal limbus, the iris, the ciliary body and the choroid were of the reactive-inflammation type, but were much milder and disappeared comparatively sooner than those in the retina. The changes in the retina were not inflammatory but degenerative and the layer of rods and cones and the outer nuclear layer were the worst affected parts. Pigment particles seemingly produced by rapid dissolution of the nuclei in the outer nuclear layer were found deposited mesh-wise in the retina, the ganglion cells and the nuclei of the inner nuclear layer were degenerated to non existence, the retina is finally atrophied down to some Müller's fibres and glia cells, which tend to adhere to the choroid. It was noticeable, that the cornea was little affected although the other tissues were subject to marked changes.

After injection of α-ch into the anterior chamber, however, the ocular tissues were never found to be seriously affected. After intravitreous injection, even the smallest dosis of 0.002 mg was found to cause perceptible damages to the retina and the ciliary processes, but the hundredfold dosis of 0.2 mg, when injected intracamerally, caused no notable lesion in any tissue of the eye. This marked difference in the detrimental effect by difference of the site of injection is perhaps mainly due to the rapid outflow from the eye-ball of the solution injected into the anterior chamber, but it may suggest also, that the cornea and iris are far more resistant than the retina and ciliary process against α-ch and that the drug introduced into the anterior chamber is not easily diffused into the vitreous body.

In the foregoing, I have described the results of my experiments for the possible injuries to the ocular tissues following injection of α-ch solution. The dosis of 0.02 mg and above were found to cause considerable damages to the retina of rabbits, if injected into the vitreous body, clearly testifying that this drug is not at all harmless to the ocular tissues. In clinical practice, the injected solution may be deemed to remain in the posterior chamber in the most part and only little permeate into the vitreous body, while the time the drug is allowed to take effect may be short. Besides, the drug causes little, if any, damage to the ocular tissues even at the higher concentration of 2 mg/cc if injected into the anterior chamber, it may be inferred that at routine clinical application the drug is free of perceptible effect on the ocular tissues other than the zonule. At operation for cataract, however, we must take into account also

the surgical attacks of incision on the cornea, the excision of the lens, iridectomy, corneal suture and such severe damages inflicted at the same time with the mere drug injection, and admit the possibility of the above-described damages coming forth more virulently than in my experiments. Especially, when the vitreous body is escaped, we must infer, that the drug solution will diffuse unchecked in the vitreous body, so that the effect of the drug would probably appear much more severely. Therefore, at clinical application of α-ch, it is of course imperative to pay consideration to the ocular troubles observed in the above experiments.

Conclusions

1. With the aim of obtaining informations on the possible untoward effects of α-chymotrypsin on the ocular tissues, intravitreously 0.002, 0.02, 0.2 and 2 mg and intracameraly 0.02 and 0.2 mg of the drug dissolved in 0.1 cc of physiological saline was injected into the normal white rabbits eyes and the results were histologically examined.

2. Injection of α-ch into the vitreous body, in the low dosis of 0.02 mg already, caused more or less severe changes in all the ocular tissues, of which the damages to the retina were particularly serious.

3. In the bulbar conjunctiva, the corneal limbus, the iris, the ciliary body and the choroid, the injection caused reactive inflammation, which, however, was far milder and faded out sooner than the damages caused in the retina.

4. The retina was subject mainly to degenerative, but little to inflammatory changes, and the layer of rods and cones and the outer nuclear layer of it were particularly sensitive to the effect. Pigment particles apparently produced by rapid karyolysis in the outer nuclear layer were deposited mesh-wise on the retina, the ganglion cells and the nuclei of the inner nuclear layer were degenerated and annihilated, and finally the retina fell into atrophy, Müller's fibres and glia cells only remaining to the last, and the tendency of adhesion to the choroid was in evidence.

5. It was noteworthy, that the cornea was little affected in any case, despite such prominent lesions in the other ocular tissues.

6. When introduced into the anterior chamber, even 0.2 mg of the drug caused no perceptible permanent damages to the ocular tissues.

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STUDIES ON THE RIBOFLAVIN METABOLISM IN EPIDEMIC KERATOCONJUNCTIVITIS

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It has been proved that E.K.C. (Epidemic Keratoconjunctivitis) is caused by Adenovirus Type 8.[1)2)] There have been many studies on E.K.C.,[3)-12)] but none of them from the view point of riboflavin (VB_2) metabolism. The present work was undertaken in an attempt to investigate the riboflavin metabolism in E.K.C. and the effects of vitamin B_2 on E.K.C., especially on the appearance of K.S.P. (Keratitis Superficialis Punctata).

Those studies, in 128 cases of E.K.C., consist of three parts:

Part A. The total riboflavin concentration in blood at the first appearance of K.S.P.; in 48 cases

Part B. The clinical course and riboflavin metabolic disturbance in E.K.C.; in 34 cases

Part C. The effect of VB_2 (3 forms: FAD, FMN, FR) on K.S.P. and the changes of riboflavin concentration in blood of E.K.C. treated with VB_2; in 46 cases

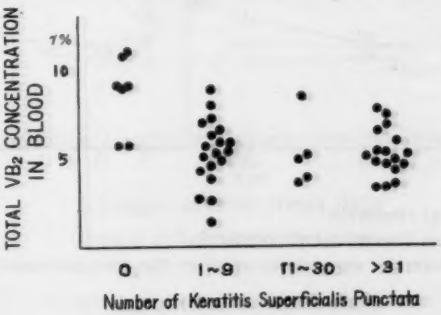
Method

The lumiflavin microfluorophotometry (Fujita's method[13)]) was used to measure riboflavin concentration in blood and the bound form was determined by benzylalcohol.

Part A. This study was carried out in 48 cases of E.K.C., at the first appearance of K.S.P. or about two weeks after onset of the infection. (Group A) (Fig. 1)

Part B. The clinical course in 34 cases of E.K.C. which were encountered in our clinic and did not receive any local treatment of VB_2 was investigated in detail for about a month. (Group B) (Fig. 2,3,4)

Fig. 1 VB_2 metabolism in Epidemic Keratoconjunctivitis (E.K.C.)
Total riboflavin concentration in blood at the beginning of Keratitis Superficialis Punctata(K.S.P.); (Group A)



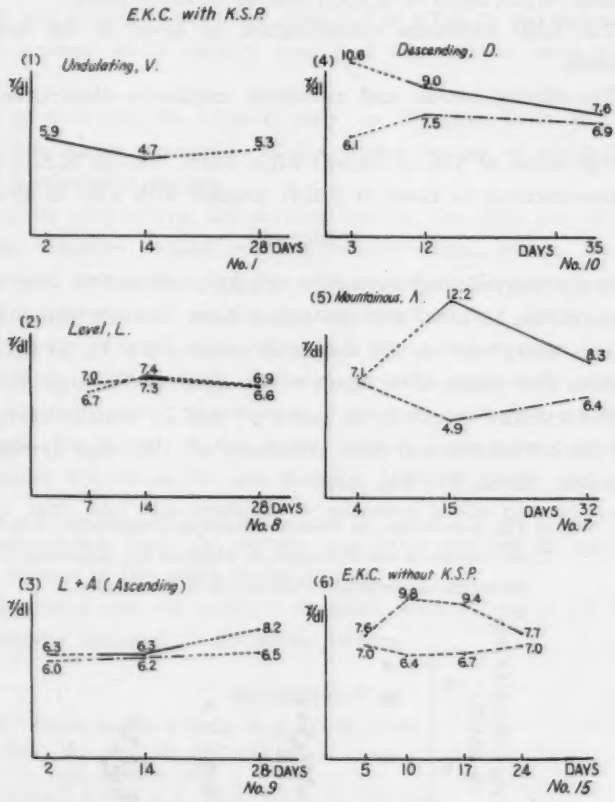
Riboflavin metabolic disturbance was ascertained by fluorophotometric measurement of the riboflavin concentration in blood, at three times during the course of E.K.C.; that is, at the beginning of conjunctivitis, at the onset of K.S.P., and 30–60 days after the beginning of conjunctivitis. (Fig. 2,3,4) (Table. 1)

Part C. The effects of VB_2 (3 forms; FAD, FMN, FR) were investigated in 46 cases of E.K.C. (group C) before and after the appearance of K.S.P.. VB_2 was administered through various methods; (Table 2,3)

local use (instillation, ointment, and subconjunctival injection)

systemic use (internal use and parenteral injection)

Fig. 2. Various forms of riboflavin concentration curve in E.K.C. (Group B).



—— Total riboflavin

– – – Ester (bound form) riboflavin

---- Before the appearance or after the disappearance of K.S.P.

Moreover, the changes of riboflavin concentration in blood in the 46 cases of E.K.C. treated with VB_2 were observed, and the character of the occurrence of K.S.P. was studied. (Fig. 5)

The application form and dose of VB_2 (3 forms) is as follows.

I. Dose of local therapy

Fig. 3. VB_2 metabolism in EKC. with K.S.P.

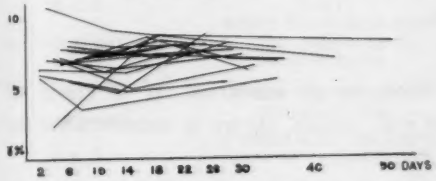


Fig. 4. VB_2 metabolism in E.K.C. without K.S.P.

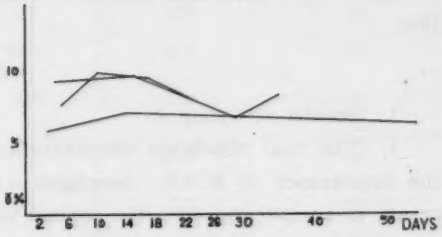
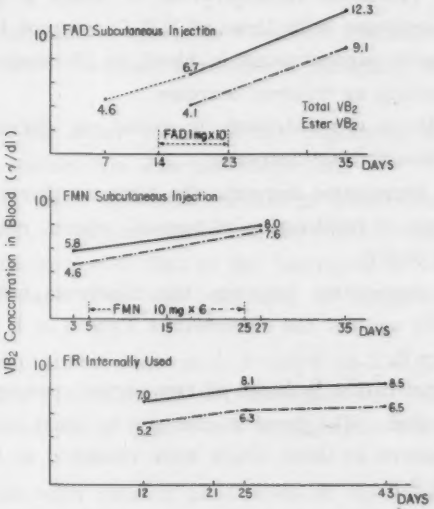


Fig. 5. The influence of VB_2 (3 forms) applied to riboflavin concentration in blood (Group C)



1. FAD local use

1) subconjunctival injection 1 mg/cc 0.1-0.3 mg(100γ-300γ)every day or every other day.

2) instillation 1.2 mg/cc several times daily

3) ointment 1 mg/g 2-3 times every day

2. FMN local use

instillation 0.7 mg/cc several times daily

II. Dose of systemic therapy

1. FAD subcutaneous injection 1-5 mg every day
2. FMN 〃 〃 10 mg every day
3. FR internal use 10-20 mg every day

cf. FAD: flavin-adenine-dinucleotide $C_{27}H_{33}O_{15}N_9P_2$
FMN; flavin-mononucleotide $C_{17}H_{21}O_9N_{24}P$
FR: free riboflavin $C_{17}H_{20}O_6N_4$

Those 124 cases of E.K.C. were encountered in our clinic from Sept. 1957 to Nov. 1958.

Results

I Results of Group A.

1. The toal riboflavin concentration in blood, in 41 cases out of 48 cases at the appearance of K.S.P., averages 5.27 ± 0.57 γ/dl, which shows a considerable decrease in comparison with the normal value. (Fig. 1)

. The total riboflavin concentration in blood in 7 cases of E.K.C. without K.S.P. averages 8.70 ± 1.91 γ/dl, which shows a normal value.

3. Thus the total riboflavin concentration in blood in cases with K.S.P. shows marked decrease in comparison with those of E.K.C. without K.S.P..

4. The total riboflavin concentration in blood, in 23 cases of severe conjunctivitis, averages 6.0 γ/dl, presenting an evident decrease.

5. The total riboflavin concentration in blood, in corneal ulcer and keratitis filamentosa showed a considerable decrease.

6. There was no correlation between the total riboflavin concentration in blood, and the form, size, grade or localization of corneal edema, pericorneal swelling, and limbal vessel-change of K.S.P..

7. There was no correlation between the riboflavin concentration in blood and the neutralizing antibody against the Adenovirus Type 8 in E.K.C..

II Results of Group B.

The riboflavin concentration in blood of two normal persons were almost constant during a one-month period. The form of changes in blood riboflavin concentration in 34 cases of E.K.C. measured at three times were classified as follows:

1. 29 cases with K.S.P.

lst type	undulating type (V-type)	13 cases
2nd 〃	level type (L-type)	8 〃
3rd 〃	descending type (D-type)	2 〃
4th 〃	ascending type (A-type)	1 case
5th 〃	mountainous type (Λ-type)	5 cases

2. 5 cases without K.S.P.

The first and the third type indicate, that a severe riboflavin metabolic disturbance

Table 1.

Cases	Periods	Total B_2 γ/dl	Ester B_2 γ/dl	Ester/Total:Ratio %
24 cases of E.K.C. with K.S.P. (V+D type)	On the appearance of conjunctivitis	7.8±0.97	6.3±1.52	81 (19~103)
	On the appearance of K.S.P.	6.4±0.96	5.9±0.85	92 (53~106)
	within 30~60days since the beginning of conjunctivitis	7.5±0.97	6.7±0.87	89 (79~115)
5 cases of E.K.C. (A type)	On the appearance of conjunctivitis	5.3	4.9	92
	On the appearance of K.S.P.	10.1	6.7	66
	within 30~60 days since the beginning of conjunctivitis	6.7	5.6	84
5 cases of E.K.C. without K.S.P.	On the appearance of conjunctivitis	7.2	5.3	74
	2 weeks after the appearance of conjunctivitis	8.4	7.3	87
	at the end of conjunctivitis	7.4	5.2	70

occured at the beginning of K.S.P..

Among the cases of the second type, the riboflavin concentration in blood was very low in 5 out of the 8 cases. Therefore a riboflavin metabolic disturbance occured to some extent.

In the fourth type, the ester ratio at the beginning of K.S.P. was very low. Therefore the riboflavin metabolism was also disturbed.

From another point of view, the total riboflavin concentration in blood in the 24 cases of type 1,2,3 and 4 at the beginning of K.S.P. (6.4±0.96 γ/dl) showed an evident decrease in comparison with that at the beginning of conjunctivitis (7.8±0.92 γ/dl) and with that 30-60 days after the beginning of conjunctivitis (7.5±0.97 γ/dl) (Fig. 3).

In cases of the mountainous type and 5 cases of E.K.C. without K.S.P., the total riboflavin concentration in blood did not decrease, but increased at the beginning of K.S.P., or in the absence of K.S.P. two weeks after the beginning of conjunctivitis.

On the other hand the ester ratio of all 5 cases of the mountainous type showed an evident decrease at this time.

However the ester ratio of 5 cases without K.S.P. did not decrease, but increased. (Fig. 4)

III Results of Group C.

The results as to the possible curative effect of VB_2 in E.K.C. is as follows:

1. The effect of VB_2 on corneal opacity in K.S.P.

1) The effect of FAD treatment (Table 2,3)

Table 2.

	E.K.C. (Group C)	Before the appearance of K.S.P.												After the appearance of K.S.P.											
		VB_2 treatment		FAD					FMN					FR					FAD					FMN					FR				
	Application	Number of corneal opacities →		Unknown	—	1 | 5	6 | 15	16 | 30	31 ∧	—	1 | 5	6 | 15	16 | 30	31 ∧	—	1 | 5	6 | 15	16 | 30	31 ∧	Unknown	—	1 | 5	6 | 15	16 | 30	31 ∧	—	1 | 5	6 | 15	16 | 30	31 ∧	—	1 | 5	6 | 15	16 | 30	31 ∧
2 weeks later	Local	subconjunctival injection		●		●●	●●	●●	●											●●	●●					×								
		instillation ointment				●	●●													●●	●●	●●	●											
	General	subcutaneous injection				×	×	●			○												●●		○	○					○	○		
		Per os													○	○	○	○					×											
	Local + General	FAD	subconj. + FMN Subcutan. Subconj. + FR Per os					●															●											
			Nicotinic acid			●																												
		FMN+FR									○	○													○									
4 weeks later	Local	subconjunctival injection		●		●●	●●	●●	●											●●	●●					×								
		instillation ointtment				●	●●													●●	●●	●●	●											
	General	subcutaneous injection				×	×	●			○												●●		○	○					○	○		
		Per os													○	○	○	○					×											
	Local + General	FAD	subconj. + FMN subcutan. Subconj. + FR Per os					●															●											
			Nicotinic acid			●																												
		FMN+FR									○	○													○									

● FAD : ○ FMN, FR : × FAD+FMN

i) The Group of 22 patients who had been locally treated with FAD, before and after the first appearance of K.S.P., showed a significant decrease in the number of corneal opacities at the maximum stage of K.S.P., compared with 17 patients who underwent the natural course of E.K.C. without any VB_2 treatment. (Table 3-a)

ii) (a) In 15 cases treated locally with FAD, especially 8 cases which received subconjunctival injection after the first appearance of K.S.P., the number of corneal opacities was decreased two weeks after the first appearance of K.S.P.. However, the number of corneal opacities four weeks after its first appearance did not differ in a statistically significant way from that during the natural course of E.K.C.. (Table 3-b)

(b) In 7 cases with FAD subconjunctival injection before the first appearance of K.S.P., the number of corneal opacities was decreased two and four weeks after the first appearance of K.S.P.. (Table 3-b)

iii) In 7 cases of local application with FAD instillation and ointment before and after the first appearance of K.S.P., the number of corneal opacities tended to decrease at the maximum stage, two and four weeks after the first appearance of K.S.P., compared with the natural course of E.K.C. (Table 3-c)

iv) In 6 cases treated systemically with FAD by subcutaneous injection, the number of corneal opacities showed a tendency to decrease two and four weeks after the first appearance of K.S.P., compared with the natural course of E.K.C.. (Table 3-d)

v) The first eye treated locally with FAD, recovered earlier than the eye without VB_2 treatment. The local application of FAD was very effective to the eye with the severe epithelial defect.

2) The effect of FMN treatment

i) In 2 cases treated locally with FMN by instillation after the appearance of K.S.P., the number of corneal opacities did not decrease.

ii) When FMN has been used systemically by subcutaneous injection before and after the appearance of K.S.P., the number of corneal opacities was decreased two and four weeks after the first appearance of K.S.P., (Table 3-e)

3 The effect of FR treatment

In 10 cases treated with FR given internally before and after the appearance of K.S.P., the number of corneal opacities did not show a significant decrease in comparison with the natural course of E.K.C.. (Table 3-f)

2. The effect of VB_2 on the riboflavin metabolism

The local application of FAD in 22 cases and FMN in 2 cases did not raise nor alter the riboflavin concentration in blood. However the subcutaneous injection of FAD in 6 cases and FMN in 7 cases were very effective in raising the abnormally low blood levels. The internal use of FR in 10 cases was sometimes effective and sometimes not very effective.

Table 3.

2 Weeks after KSP	Natural course	After the appearance of KSP	Before the appearance of KSP
0	4	4	5
1~5	4	3	4
over 6	9	5	1

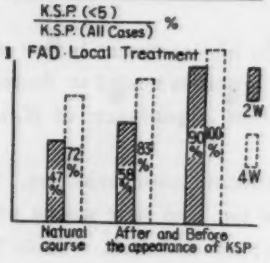
2 W. after KSP	Natural course	After the appearance of KSP	Before the appearance of KSP
0	4	3	4
1~5	4	2	2
over 6	9	3	1

2 W. after KSP	Natural course	After the appearance of KSP	Before the appearance of KSP
0	4	1	1
1~5	4	1	2
over 6	9	2	0

4 W.	Natural	After	Before
0	5	5	9
1~5	3	5	1
over 6	3	2	0

4 W.	Natural	After	Before
0	5	3	7
1~5	3	3	0
over 6	3	2	0

4 W	Natural	After	Before
0	5	2	2
1~5	3	2	1
over 6	3	0	0



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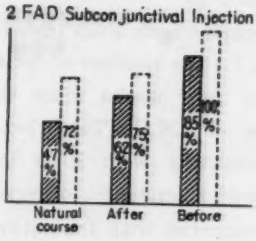

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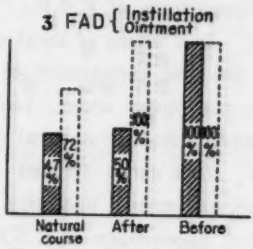


c

2 Weeks after KSP	Natural course	After the appearance of KSP	Before the appearance of KSP
0	4	0	2
1~5	4	0	1
over 6	9	3	0

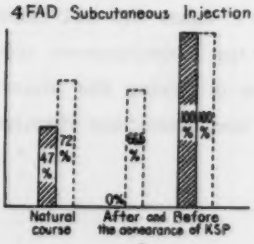
2 W. after KSP	Natural course	After the appearance of KSP	Before the appearance of KSP
0	4	1	4
1~5	4	1	1
over 6	9	0	0

2 W. after KSP	Natural course	After the appearance of KSP	Before the appearance of KSP
0	4	0	2
1~5	4	1	2
over 6	9	2	3

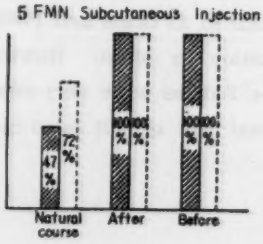
4 W.	Natural	After	Before
0	5	0	2
1~5	3	2	1
over 6	3	1	0

4 W.	Natural	After	Before
0	5	2	4
1~5	3	0	1
over 6	3	0	0

4 W.	Natural	After	Before
0	5	0	3
1~5	3	2	1
over 6	3	1	3



d



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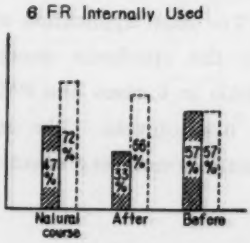

f

The forms of changes in blood riboflavin concentration in all 15 cases of FAD therapy by subconjunctival injection, were almost undulating, or descending type.

The riboflavin concentration in all 7 cases of FAD ointment and instillation therapy was rather low, showing, that the riboflavin metabolism was still disturbed.

Discussion

VB_2 has been proved to be very effective in the treatment of many corneal diseases by enhancing the metabolism of corneal epithelium. There have been many [14-19] reports about the curative effect of VB_2 in various corneal diseases.

However, there have been only few reports which have made the mechanism of VB_2 effectiveness clear, except for that of, Funatsu who utilized biochemical methods.

Funatsu[20] concluded, that only a local use of FAD seemed to be worth-while clinically and the subconjunctival injection of FAD appeared to have a clinical significance as far as the corneal metabolism is concerned.

I have studied the relation between the riboflavin metabolic disturbance and the course of E.K.C., especially the appearance of K.S.P.. From the results of my experiments, I have noticed many interesting facts such given below. as follows:

1. The fact that the total riboflavin concentration in blood had decreased in 41 cases at the time of K.S.P. appearance indicates, that there should be $V.B_2$ deficiency at the first appearance of K.S.P. in the course of E.K.C..

2. On the contrary, the fact, that the total riboflavin concentration in blood did not decrease in 7 cases of E.K.C. without K.S.P. means that K.S.P. may not appear in the cases of E.K.C. with normal riboflavin metabolism.

3. Therefore such an abnormal riboflavin metabolism is assumed either to have existed before the infection, or to be caused by or after the infection of E.K.C..

From these results I have noticed and assumed as follows.

1. Those who have the riboflavin metabolic disturbance are apt to develop K.S.P. during an infection of E.K.C..

2. Even the cases of no riboflavin metabolic disturbance are apt to have a decreased blood level of riboflavin at the time of the first appearance of K.S.P. on the infection of E.K.C..

There have been many[21)-24)] reports that riboflavin metabolism is disturbed in various diseases, which are classified into two types as follows.

1. Type of chronic VB_2 deficiency (Thyroid disease, tuberculosis) and VB_2 metabolic insufficiency (diabetes mellitus, hypophyseal disease, chronic gastric disease, hypertension, acidosis)

2. Type of acute (temporal) VB_2 deficiency (alimental deficiency, pregnancy, morbus basedowii, growing age)

However, riboflavin metabolic disturbance on the course of E.K.C. slightly differs from those disturbances.

As Rewerts[25] reported, riboflavin metabolism is disturbed in acute infectious diseases.

Sugiura[1] has established that E.K.C. is an acute infectious disease due to Adenovirus Type 8. Hanna[26], Jawetz[27], Mitsui[28], et al had isolated the Adenovirus Type 8 from the epithelial cell of the mucous membrane of palpebral conjunctiva of the patients with E.K.C..

Hilleman and[29], Fnkumi[30] had isolated the virus from the nostrilpharyngeal mucous membrane, Kjellen[31] had found it in a mesenchymal lymphatic gland.

In most cases, riboflavin metabolic disturbance is apt to appear during the course of E.K.C., especially at the beginning of K.S.P., and in author's opinion, the form of the metabolic disturbance belongs to the defects in utilizing VB_2 in the intestine (FR-FMN) caused by the infection of the Adenovirus Type 8.

As to the curative effect of VB_2 in E.K.C., I have obtained the following results.

1. The local application of FAD, especially subconjunctival injection before and after the appearance of K.S.P., was very effective in the cure of corneal opacities two weeks after the appearance of K.S.P., in comparison with the natural course of E.K.C..

2. The systemic use of FAD was effective in the cure of corneal opacities and improving the metabolic disturbance of riboflavin.

3. The local application of FMN was not effective in the cure of corneal opacities, but the systemic use of it was effective to cure the corneal opacities and improve the metabolic disturbance of riboflavin.

4. When the systemic application and subconjunctival injection of FAD were combined, the cnre of K.S.P. was more efficient than the separate application of either. (Fig. 5)

5. The systemic application of FR was sometimes effective and sometimes not very effective.

Thus, the local and systemic application of FAD was very effective. The local application of FMN was not effective. On the other hand the systemic application of FR was relatively effective.

The reason why FR is not very effective in the course of E.K.C. is due to the riboflavin metabolic disturbance, which lies chiefly in the difficulty of the synthesis of FMN from FR in the intestines, brought about by the infection of Adenovirus Type 8.

Summary and Conclusion

1. The toal riboflavin concentration in blood in cases of K.S.P. shows an evident decrease in comparison with cases of E.K.C. without K.S.P., and this indicates that there is some relation between the riboflavin metabolic disturbance and the mechanism of the appearance of K.S.P..

2. Through the fact that there are five forms of riboflavin concentration curves in E.K.C. it is assumed, that an utilizing disturbance of riboflavin metabolism may occur in the course of E.K.C..

3. 1/ During the course of E.K.C., the subcutaneous injection of FAD or FMN was very effective to correct the depressed blood levels of riboflavin.

2/ To decrease the number of corneal opacities the local application of FAD was quite powoerful.

3/ When FAD was applied locally by subconjunctival injection combined with systemic use, and when FMN was given systemically, the cure of K.S.P. was morecal efficient.

4/ The local application of FMN was not effective.

5/ The systemic application of FR was sometimes effective, and sometimes not very effective. The reason why FR is not very effective is assumed to come from the riboflavin metabolic disturbance during the course of E.K.C., which lies chiefly in the difficulty of the synthesis of FMN from FR in the intestines, brought about by the infection of Adenovirus Type 8. (The method of R.A. Fisher was used for statistical-evaluation.)

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STUDIES ON THE NICOTINIC ACID METABOLISM AT VARIOUS EYE DISEASES

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Introduction

In the field of ophthalmology there are numerous studies regarding riboflavin, while one can find hardly those of nicotinic acid belonging to the vitamine B_2 complex. Thereupon, inquiries were first made into the serial fluctuation and the normal value of nicotinic acid metabolism and riboflavin metabolism in the healthy body. Then inquiries were made into superficial diffuse keratitis, the cause of which had been considered to be riboflavin deficiency, blepharitis supposed to be caused by riboflavin deficiency, vitamine B_6, etc., Behçet's syndrome, Morbus Haradai and uveitis, the causes of which had been supposed to be metabolic disorder in riboflavin and nicotinic acid, among various eye diseases, and also into the relation to riboflavin metabolism. That is, nicotinic acid and riboflavin in the blood and nicotinic acid, N′-methylnicotinamide, N′-methyl-3-carboxylamide-6-pyridone and riboflavin in urine calculated simultaneously in the same materials.

Materials and Methods

Materials. As for serial fluctuation the researches were made on two healthy men. The normal value in a healthy body was measuured in 12 men and 12 women. As for the patients of eye diseases, 16 cases of blepharitis, 10 cases of superficial diffuse keratitis, 10 cases of blepharitis with superficial diffuse karatitis, 12 cases of Behçet's syndrome, 5 cases of Morbus Haradai and 5 cases of uveitis.

Standard Solution. Nicotinic acid made by Eastman Kodak was used. As for N′-methylnicotinamide, N′-methylnicotinamide-iodide (m.p. 203–204°C) methylated nicotinamide (made by Daiichi Chemistry) by the Holman method was used. N′-methyl-3-carboxylamide-6-pyridone (m.p. 127°C) synthesized by the Huff method was used. As for riboflavin, riboflavin crystal made by Merck was used.

Methods

(a) Nicotinic Acid. Nicotinic acid was estimated by a modification of tke Kawa

shima (1947) & Nagayama (1955) method (Katsura 1953).

(b) N′-methylnicotinamide. This was estimated by a modification of the Huff (1947) method (Nose 1950).

(c) N′-methyl-3-carboxylamide-6-pyridone. This was estimated by a modification of the Rosen (1949) method (Tomabeji 1952).

(d) Riboflavin. The total riboflavin and its bound form were calculated by a technique of lumiflavinfluorophotometry (Fujita 1951).

Results and Discussion

On the nicotinic acid metabolism in the healthy body. Researches were made on serial and individual fluctuations of the nicotinic acid metabolism in a healthy body by measuring the nicotininic acid level in the blood and the excretion of nicotinic acid, N′-methyl-nicotinamide and N′-methyl-3-carboxylamide-6-pyridone in urine.

1. The average nicotinic acid level in the blood (12 men and 12 women) was 0.46±0.080 mg/dl, and the individual or serial fluctuation was rather small.

2. The average excretion of nicotinic acid in urine per day (12 men and 10 women) was 2.4±0.71 mg, which had some relation with the nicotinic acid level in the blood, and it showed less individual fluctuation than that of N′-methylnicotinamide and N′-methyl-3-carboxylamide-6-pyridone.

3. The average excretion of N′-methyl-nicotinamide in urine per day (12 men and 10 women) was 4.6±1.47 mg and that of N′-methyl-3-carboxylamide-6-pyridone (12 men and 6 women) was 11.8±2.30 mg. The individual and dietary fluctuations was remarkable.

4. Proportion of excretion of the three products from nicotinic acid metabolism in urine was estimated as 100 : 192 : 492 for nicotinic acid : N′-methylnicotinamine : N′-metyl-3-carboxylamide-6-pyridone.

5. The average total excretion of micotinic acid metabolism products (the grand total of the nicotinic acid excretion and those which have been converted equivalently from N′-methylnicotinamide and N′-methyl-3-carboxylamide-6-pyridone, herein called T. T=excretian of nicotinic acid+excretion of N′-methylnicotinamide×0.714+excretion of N′-methyl-3-carboxylamide-6-pyridone×0.8095) in urine per day (12 men and 6 women) was 15.3±2.56 mg.

On the correlation between nicotinic acid metabolism and riboflavin metabolism in a healthy body and on the serial fluctuation of riboflavin metabolism.

Researches were made on serial and individual fluctuations of the riboflavin metabolism in a healthy body by measuring the total riboflavin and its bound form in the blood, and the excretion of riboflavin in urine also. Furthermore, the correlation between nicotinic acid and riboflavin metabolism has been studied.

1. The average total riboflavin level in the blood (12 men and 11 women) is 8.0±1.46 μg/dl, fluctuation of which is rather small both individually and serially through a year.

2. The average level in the blood of the bound form of riboflavin (10 men and 11 women) is 6.5±1.11 μg/dl, fluctuation of which is small individually or serially, but through year some fluctuations are noticed. Further research on this subject is intended to be made id the near future.

3. The average excretion of riboflavin in urine per day (11 men and 7 women) is 1382±603 μg, and its serial fluctuation is comparatively small, while individual or serial fluctuation through a year is found to be remarkable.

4. The total excretion of nicotinic acid metabolism indicated less excretion in the urine of the group who excrete much riboflavin per day, while it indicates much in the group that excretes less riboflavin.

On the nicotinic acid metabolism at various eye diseases. Researches were made on the nicotinic acid metabolism at various eye diseases—blepharitis, superficial diffuse keratitis, blepharitis with superficial diffuse keratitis, Behçet's syndrome, Morbus Haradai, uveitis, etc.—and, at the same time, its relation to the riboflavin metabolism.

1. Blepharitis (Table 1, 2)

Table 1. Nicotinic Acid Metabolism in Blepharitis

Case No.	Sex	Age	Diagnosis	Nicotinic Acid level in blood mg/dl	Excretion in urine per day				urinevolume cc
					Nicotinic Acid mg	N'-methylnicotinamide mg	6-pyridone * mg	T. mg	
1	♂	46	marginal blepharitis	0.49	2.3	9.6	12.3	19.2	985
2	♂	53	〃	0.55	1.4	2.1	8.0	9.4	640
3	♀	27	〃	0.43	3.1	0.7	5.6	8.1	750
4	♂	62	〃	0.46	4.9	6.8	11.0	18.6	1470
5	♂	23	〃	0.54	1.8	6.8	1.7	8.1	735
6	♂	55	〃	0.32	2.7	5.0	6.0	11.2	1335
7	♀	15	〃	0.21	—	—	—	—	—
8	♀	9	〃	0.26	0.3	0.7	2.6	2.1	850
9	♀	12	〃	0.45	1.2	10.3	10.5	17.1	1165
10	♀	8	〃	0.41	0.7	0.7	1.6	2.5	520
11	♀	17	〃	0.34	—	—	—	—	—
12	♀	18	〃	0.21	—	—	—	—	—
13	♀	9	〃	0.33	—	—	—	—	—
14	♂	21	eczematous blepharitis	0.50	2.4	2.8	6.6	9.8	1315
15	♂	21	angular blepharitis	0.38	1.1	4.6	7.9	10.8	900
16	♀	22	〃	0.52	2.7	2.6	8.3	11.3	1380
average 99% confidential limit				0.40 ±0.082	2.1 ±1.04	4.4 ±3.02	6.8 ±3.19	10.7 ±4.95	
average in marginal blepharitis 99% confidential limit				0.38 ±0.112	2.0 ±1.65	4.7 ±4.38	6.6 ±4.60	1.07 ±7.21	

* 6-pyridone=N'-methyl-3-carboxylamide-6-pyridone

Table 2. Riboflavin Metabolism & Complication in Blepharitis

Case No.	Sex	Age	Diagnosis	Level in blood: total μg/dl	Level in blood: bound form μg/dl	Level in blood: bound form / total Ratio %	Excretion in urine per day μg	Conjunctivitis	Pericorneal capillarisation	Glossitis	Cheilitis	Angular Stomatitis
1	♂	46	marginal blepharitis	6.7	5.3	79.1	481	−	−	−	−	−
2	♂	53	〃	7.5	6.7	89.3	313	−	−	+	+	+
3	♀	27	〃	8.9	6.7	75.3	934	−	−	+	+	−
4	♂	62	〃	9.8	5.7	58.2	449	−	−	−	−	−
5	♂	23	〃	7.6	7.3	96.1	1050	−	−	−	−	+
6	♂	55	〃	8.7	7.0	80.5	2814	−	±	−	−	−
7	♀	15	〃	9.2	6.9	75.0	−	+	−	−	−	−
8	♀	9	〃	9.1	6.5	80.2	561	−	−	−	−	−
9	♀	12	〃	6.3	5.8	92.1	4349	+	−	−	+	−
10	♀	8	〃	7.2	−	−	141	+	−	−	−	−
11	♀	17	〃	7.3	6.8	93.2	−	−	−	−	+	−
12	♀	18	〃	−	−	−	−	−	−	−	+	−
13	♀	9	〃	−	−	−	−	−	−	−	−	−
14	♂	21	eczematous blepharitis	5.4	−	−	480	−	−	−	−	−
15	♂	21	angular blepharitis	9.2	5.5	59.8	532	+	−	−	−	−
16	♀	22	〃	6.3	6.3	100.0	2588	−	−	−	−	−
Average 99% confidential limit				7.7 ±1.19	6.4 ±0.24		1224 ±1176					
Average in marginal blepharitis 99% confidetial limit				7.9 ±1.30	6.5 ±0.67		1237 ±1577					

The average nicotinic acid level in the blood (7 men and 9 women) was 0.40±0.082 mg/dl. The average excretion of nicotinic acid in urine per day (7 men and 5 women) was 2.1±1.04 mg. The average excretion of N′-methylnicotinamide in urine per day (7 men and 5 women) was 4.4±3.02 mg and that of N′-methyl-3-carboxylamide-6-pyridone (7 men and 5 women) was 6.8±3.19 mg. The average total excretion of nicotinic acid metabolism products in urine per day was 10.7±4.95 mg. In comparison with the normal value, in blepharitis excretion of N′-methyl-3-carboxylamide-6-pyridone only decreased with a significant difference of 1% level. In so far as only marginal blepharitis is concerned, the average nicotinic acid level in the blood (5 men and 8 women) showed at 0.38±0.112 mg/dl, presenting an evident decrease with a significant difference of 1% level also. Accordingly it was considered, that in marginal blepharitis the nicotinic acid metabolism was abnormal but riboflavin metabolism was normal.

2. Superficial diffuse heratitis (Table 3,4).

The average nicotinic acid level in the blood (2 men and 8 women) showed to be at 0.29±0.125 mg/dl, presenting an evident decrease with a significant difference of 1% level. The average excretion of nicotinic acid in urine per day (2 men and 6 women) showed to be at 1.6±0.75 mg, presenting a decrease with significant difference of 10%

Table 3. Nicotinic Acid Metabolism in Superficial Diffuse Keratitis

Case No.	Sex	Age	Nicotinic Acid level in blood mg/dl	Excretion in urine per day				Urinevolume cc
				Nicotinic Acid mg	N'-methyl-nicotinamide mg	6-pyridone mg	T mg	
1	♀	47	0.43	1.2	4.0	—	—	1490
2	♀	31	0.20	1.6	5.3	10.4	13.8	1390
3	♀	58	0.14	0.8	2.5	7.2	8.4	800
4	♀	31	0.28	1.0	6.7	9.7	13.6	810
5	♀	29	0.27	2.1	0.6	5.4	6.9	1785
6	♂	20	0.57	2.1	2.9	10.4	12.6	1725
7	♀	53	0.28	2.2	11.1	—	—	2250
8	♂	31	0.32	2.0	5.3	3.6	8.7	1185
9	♀	37	0.15	—	—	—	—	—
10	♀	51	0.29	—	—	—	—	—
Average 99% confidential limit			0.29 ±0.125	1.6 ±0.75	4.8 ±3.78	7.8 ±4.72	10.7 ±4.97	

Table 4. Riboflavin Metabolism & Complication in Superficial Diffuse Keratitis

Case No.	Sex	Age	Level in blood			Excretion in urine per day μg	Conjunctivitis	Pericorneal Capillarisation	Grossitis	Cheilitis	Angular Stomatitis
			total μg/dl	bound form μg/dl	bound form total / Ratio %						
1	♀	47	6.3	—	—	—	—	—	—	—	—
2	♀	31	6.0	—	—	449	—	+	+	—	+
3	♀	58	6.9	6.7	97.1	1514	—	—	—	—	—
4	♀	31	7.2	6.4	88.9	833	+	—	—	—	—
5	♀	29	6.6	6.0	90.9	1307	—	—	—	+	+
6	♂	20	5.1	5.5	107.8	863	+	+	—	—	—
7	♀	53	2.9	—	—	1149	—	+	+	+	+
8	♂	31	6.3	6.3	100.0	633	—	+	+	+	+
9	♀	37	—	—	—	—	—	—	—	—	—
10	♀	51	6.9	6.6	94.2	—	—	+	—	—	—
average 99% confitdential limit			6.0 ±1.60	6.3 ±0.73		964 ±529					

level and that of N'-methyl-3-carboxylamide-6-pyridone showed to be at 7.8±4.72 mg, 2% level. The average total excretion of nicotinic acid metabolism products in urine (2 men and 4 women) showed to be at 10.7±4.97 mg, presenting a decrease with significant difference of a 2% level. The average total riboflavin level in the blood (2 men and 7 women) showed to be 6.0±1.60 μg/dl, presenting a decrease with significant difference of a 5% level. Accordingly it was considered, that in superficial diffuse keratitis not only riboflavin metabolism, but the nicotinic acid metabolism was abnormal.

Table 5. Nicotinic Acid Metabolism in Blepharitis witl Superficial Diffuse Keratitis

Case No.	Sex	Age	Nicotinic Acid level in blood mg/dl	Excretion in urine per day				Urinevolume cc
				Nicotinic Acid mg	N'-methyl-nicotinamide mg	6-pyridone mg	T mg	
1	♀	16	0.32	1.4	3.8	17.3	18.1	990
2	♂	21	0.47	2.2	3.9	10.1	13.2	1350
3	♀	27	0.38	1.6	3.3	4.2	7.4	1390
4	♂	65	0.30	1.6	8.9	2.0	9.6	1345
5	♀	36	0.29	2.5	0.6	9.7	10.7	1610
6	♀	21	0.25	2.3	1.0	10.1	11.2	1680
7	♂	15	0.32	3.1	1.8	9.3	11.9	1555
8	♀	25	0.38	—	—	—	—	—
9	♀	21	0.33	2.6	3.4	14.6	16.8	650
10	♂	14	0.25	2.8	4.6	4.4	9.7	1750
average 99% confidential limit			0.33 ±0.063	2.2 ± 0.80	3.5 ±2.71	9.1 ±5.54	12.0 ±3.91	

Table 6. Riboflavin Metabolism & Complication in Blepharitis with Superficial Diffuse Keratitis

Case No.	Sex	Age	Level in blood			Excretion in urine per day μg	Conjunctivitis	Pericorneal Capillarisation	Glossitis	Cheilitis	Angular Stomatitis
			total μg/dl	bound form μg/dl	bound form / total Ratio %						
1	♀	16	7.6	4.5	59.2	268	—	—	+	+	+
2	♀	21	12.6	8.4	66.7	278	—	—	—	—	—
3	♀	27	8.2	7.5	91.5	456	—	—	—	—	—
4	♂	65	6.3	6.3	100.0	918	+	+	+	—	+
5	♀	36	8.5	6.6	77.6	1361	—	—	—	—	+
6	♀	21	6.9	6.8	98.6	642	—	+	—	—	+
7	♂	15	6.4	6.3	98.4	344	+	+	—	—	—
8	♀	25	8.7	7.1	81.6	—	+	+	+	+	—
9	♀	21	8.3	7.2	86.7	220	—	+	—	+	—
10	♂	14	9.0	5.4	60.0	144	+	—	—	+	—
average 99% confidential limit			8.3 ±1.56	6.6 ±1.19		515 ±410					

3. Blepharitis with superficial diffuse keratitis (Table 5,6)

The average nicotinic acid level in the blood (4 men and 6 women) showed to be 0.33±0.063 mg/dl, presenting an evident decrease with a significanct difference of a 1% level. The average total excretion of nicotinic acid metabolism products in urine (4 men and 5 women) showed to be 12.0±3.91 mg, presenting a decrease with a significant difference of a 5% level. Accordingly it was considered, that in blepharitis with superficial diffuse keratitis nicotinic acid metabolism was abnormal. The average riboflavin level in the blood was normal.

4. Behçet's syndrome (Table 7,8)

The average nicotinic acid level in the blood (10 men and 1 women) showed to be 0.32±0.066 mg/dl, presenting an evident decrease with a significant difference of a 1% level. The average excretion of N'-methylnicotinamide in urine per day (11 men and 1 woman) showed to be 2.1±1.97 mg, presenting an evident decrease with a significant difference of a 1% level but that of nicotinic acid (11 men and 1 woman) showed to be 3.8±1.66 mg, presenting conversely an increase with a significant difference of a 2% level. The average riboflavine level of the blood was normal. Accordingly it was considered, that in Behçet's syndrome there was nicotinic acid deficiency or nicotinic acid metabolism disturbance.

Table 7. Nicotinic Acid Metabolism in Behçet's Syndrome

Case No.	Sex	Age	Nicotinic Acid level in blood mg/dl	Excretion in urine per day				Urinevolume cc
				Nicotinic Acid mg	N'-methyl-micotinamide mg	6-pyridone mg	T mg	
1	♀	19	0.32	2.4	1.7	11.9	13.2	1185
2	♂	26	0.31	7.6	2.1	4.1	12.4	1970
3	♂	25	0.28	4.3	0.6	7.7	10.9	1135
4	♂	40	0.41	4.7	7.0	21.4	27.0	2035
5	♂	28	0.28	2.1	1.2	—	—	1500
6	♂	21	0.15	2.6	5.6	—	—	555
7	♂	28	—	4.6	0.0	13.5	15.5	1800
8	♂	55	0.37	3.0	0.1	5.0	7.1	665
9	♂	27	0.34	2.1	1.4	7.7	9.3	860
10	♂	38	0.34	2.4	0.1	5.9	7.3	1300
11	♂	34	0.34	2.5	3.4	—	—	1340
12	♂	28	0.38	7.0	1.8	—	—	1165
average 99% confidential limit			0.32 ±0.066	3.8 ±1.66	2.1 ±1.97	9.7 ±6.85	12.8 ±7.63	

5. Morbus Haradai (Table 9,10).

Not any change is noticed in both, the nicotinic acid metabolism and in riboflavin metabolism.

6. Uveitis (Table 11, 12).

The average nicotinic acid level (5 men) and total riboflavin level (5 men) in the blood showed to be 0.24±0.042 mg/dl, 6.0±1.01 μg/dl respectively, both presenting a decrease with a significant difference of a 1% level. The excretion of three nicotinic acid metabolism products in urine was normal, but that of riboflavin increased rather in comparison with the normal value.

Summary

As compared with the normal value in the healtdy body, in blepharitis excretion

Table 8. Riboflavin Metabolism & Symptoms in Behçet's Syndrome

Case No.	Sex	Age	Level in blood: total μg/dl	Level in blood: bound form μg/dl	Level in blood: bound form / total Ratio %	Excretion in urine per day μg	Aphtha	Cutaneous change	genital change	Chemotherapy
1	♀	19	7.5	6.5	86.7	593	+	−	+	+
2	♂	26	6.3	6.0	90.9	391	+	−	−	+
3	♂	25	6.0	6.0	100.0	382	+	+	+	+
4	♂	40	9.4	7.0	74.5	1647	+	+	+	−
5	♂	28	12.5	10.1	80.8	390	+	+	+	+
6	♂	21	7.3	—	—	148	+	−	−	+
7	♂	28	9.5	6.8	71.6	611	+	−	−	+
8	♂	55	9.1	7.7	84.6	1288	−	−	−	−
9	♂	27	7.1	6.1	85.9	321	−	−	−	−
10	♂	38	6.4	6.6	103.1	2474	+	−	−	+
11	♂	34	12.6	10.4	82.5	738	−	−	−	−
12	♂	28	11.1	10.6	95.5	457	+	−	+	−
average 99% confidential limit			8.7 ±2.23	7.6 ±1.85		787 ±611				

Table 9. Nictoinic Acid Metabolism in Morvus Haradai

Case No.	Sex	Age	Nicotinic Acid level in blood mg/dl	Excretion in urine per day: Nicotinic Acid mg	Excretion in urine per day: N'-methyl-nicotinamide mg	Excretion in urine per day: 6-pyridone mg	Excretion in urine per day: T mg	Urinevolume cc
1	♂	18	0.63	2.2	2.9	8.4	11.1	560
2	♂	32	0.50	1.9	3.7	20.5	21.1	1140
3	♂	48	0.40	5.4	10.9	24.6	33.1	1640
4	♀	42	0.18	2.8	0.8	8.9	10.6	1185
5	♂	50	0.32	1.7	2.4	9.0	10.7	1000
average 95% confidential limit			0.41 ±0.197	2.8 ±1.87	4.1 ±4.94	14.3 ±9.54	17.3 ±12.25	

Table 10. Riboflavin Metabolism in Morbus Haradai

Case No.	Sex	Age	Level in blood: total μg/dl	Level in blood: bound form μg/dl	Level in blood: boune form / total Ratio %	Excretion in urine per day μg
1	♂	18	8.6	7.5	87.2	1540
2	♂	32	5.7	4.9	86.0	479
3	♂	48	9.2	7.2	78.3	401
4	♀	42	6.9	6.0	89.6	1672
5	♂	50	6.9	6.4	92.8	2725
average 95% confidetial limit			7.5 ±1.39	6.4 ±1.28		1363 ±1208

Table 11. Nicotinic Acid Metabolism in Uveitis

Case No.	Sex	Age	Nicotinic Acid level in blood mg/dl	Excretion in urine per day				Urinevolume cc
				Nicotinic Acid mg	N'-metoyl-nicotinamiee mg	6-pyridone mg	T mg	
1	♂	38	0.23	3.4	7.7	3.3	11.6	2175
2	♂	36	0.28	2.9	3.0	22.6	23.3	2155
3	♂	54	0.29	1.9	3.8	12.7	14.9	1205
4	♂	17	0.14	2.3	1.2	17.9	17.7	1700
5	♂	26	0.24	2.8	4.3	20.2	22.2	1615
average 95% confidential limit			0.24 ±0.042	2.7 ±0.32	4.0 ±2.95	15.3 ±9.50	17.9 ±6.09	

Table 12. Riboflavin Metabolism in Uveitis

Case No.	Sex	Age	Level in blood			Excretion in urine per day μg
			total μg/dl	bound form μg/dl	bound form / total Ratio %	
1	♂	38	6.7	6.5	97.0	8120
2	♂	36	6.0	6.0	100.0	1773
3	♂	54	6.8	6.0	88.2	2382
4	♂	17	5.7	—	—	—
5	♂	26	4.8	5.2	108.3	—
average 95% confidential limit			6.0 ±1.01			

of N'-methyl-3-carboxylamide-6-pyridone in the urine per day showed an evident decrease, but not any other remarkable ceange was noticed. In so far as enly marginal blepharitis was concerned, however, the average nicotinic acid level in the blood also showed an evident decrease. In superficial diffuse keratitis, the average nicotinic acid level and the total riboflavin level in the blood and the total excretion of nicotinic acid metabolism products in urine showed evident decreases. In blepharitis with superficial diffuse keratitis, the average nicotinic acid level in the blood showed an evident decrease but no change in the riboflavin level in the blood. In Behçet's syndrome, a change was noticed in nicotinic acid metabolism, but not in the riboflavin level of the blood. In Morbus Haradai, not any change was noticed, both in the nicotinic acid metabolism and in riboflavin metabolism. In uveitis, an evident decrease was shown both in the nicotinic acid level and in the total riboflavin level of the blood.

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STUDY ON THE CRYSTALLIN LENS BY POLARIZED LIGHT

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The crystalline lens reveals a characteristic pattern by observing between crossed polars using a parallel or cenvergent polarized light. This pattern can be regarded as an isogyre which is considered as the interference figure. It has been reported by the previous workers, that the crystalline lens shows the uniaxial type birefringence. In the present experiment, however, it was observed as a biaxial type birefringence. As experimental specimens, the crystalline lenses of various kinds of fish (Beryx sprendens Lowe, Sebastes matsubarai Hirgendorf, etc.) and mammalia (Oryctolagus cuniculus var. Domesticus Gmelin, Bos taurus var. Domesticus Gmelin, etc.) were used.

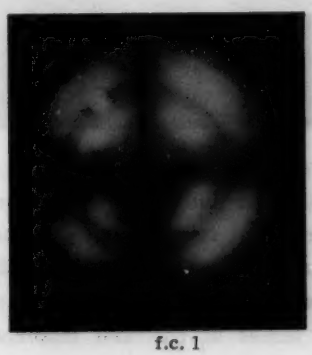
Methods and Results

1. Observation in an Isolated Intact Lens (Fig. 1).

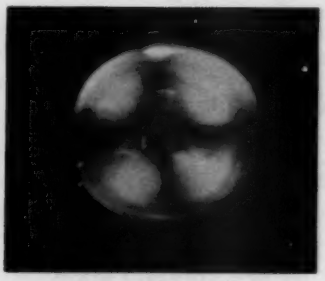
Fig. 1. Interference figure of crystalline lens.

f: fish m: mammalia s: simple type c: complex type
1: lensaxis parallel to microscopeaxis
2: lensaxis parpendicular to microscopeaxis and parallel to vibration plane of polaroid
3: lensaxis parpendicular to microscopeaxis and corresponding diagonal position of I and III quadrants
4: lensaxis parpendicular to microscopeaxis and corresponding diagonal position of II and IV quadrants

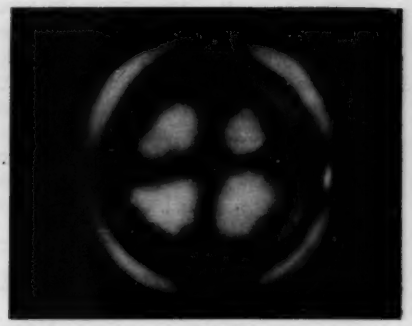
f.s. 1



f.c. 1



m.s. 1



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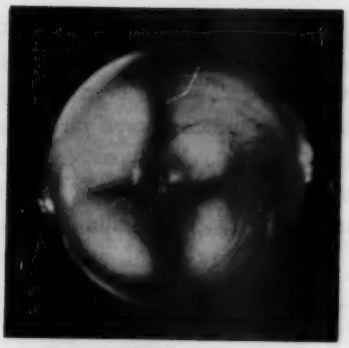
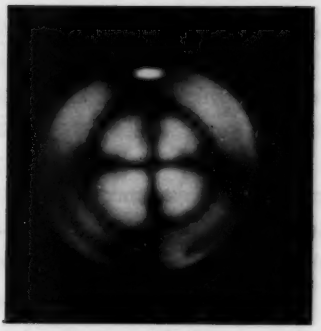


f.g. 2



f.c. 2

m.s. 2

m.c. 2

The crystalline lens was observed by a polarizing microscope with crossed polars in a parallel or convergent light from several directions. In the case of parallel light the result obtained was similar to that of the convergent light, since the crystalline lens has a convergency action by itself. The interference figures shown in the crystalline lenses of fish and mammalia were considerably various, and the patterns

differ by the directions of observing. From the polarization optical view point, however, no difference was observed fundamentally between the lenses of fish and mammalia. The interference figures showed principally two types of patterns, such as the simple and complex types. The former consists of two layers composed of thick internal and thin external layers and the latter consists of fourlayers composed of central, internal, intermediate and external layers. When a gypsum plate was superposed over the interference figure with the direction of vibration of its fast ray (X) paralleled to the diagonal position of the I and III quadrants in the simple type, the interference color of the external layer was raised, but that of the internal layer was lowered.

Fig. 1. f.s. 3

f.c. 3

m.s. 3

m.c. 3

Between these two types of interference figures, there were observed various degrees of transition by the differences specimens and families.

2. Observation in Disk-shape Specimen of the Crystalline Lens.

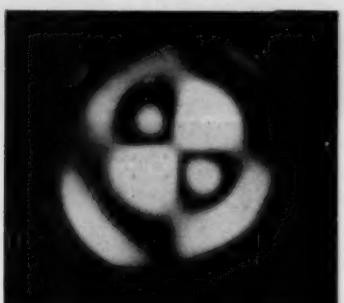
On observing the disk-shape specimen excised from the crystalline lens containing the central region the result obtained was similar to the above mentioned result. The external and intermediate layers of the complex type pattern were caused by the

cortex of the crystalline lens and the internal and central layers were caused by the nucleus. In the case of the simple type, the external layer and the outer part of the internal layer of the interference pattern were caused by the cortex.

3. Polarization-Optical Properties of Isolated Crystalline Lens Fibers.

Strips of flat lens fibers were excised from the intermediate and internal layers of the complex type and the polarization-optical properties were investigated.

f.s. 4



f.c. 4

m.s. 4

m.c. 4

Orthoscopic Observation. The relationships among the observation direction, arranging direction of fibers and vibration plane of polaroid are shown in Fig. 2. Where ↑ means the vibration plane of polaroid, and the conditions are, (A) the fibers direction is placed in parallel with the vibration plane of polaroid; (B): the fibers direction is placed in a diagonal position of I and III quadrants, and (C): the fibers direction is placed in a diagonal position of II and IV quadrants.

Conoscopic Observation. On observing the intermediate layer specimens, at (A) position, the interference figure showed a dark cross and at (B) and (C) positions, it was separated to two hyperbolas in the opposite quadrants. When a gypsum plate is

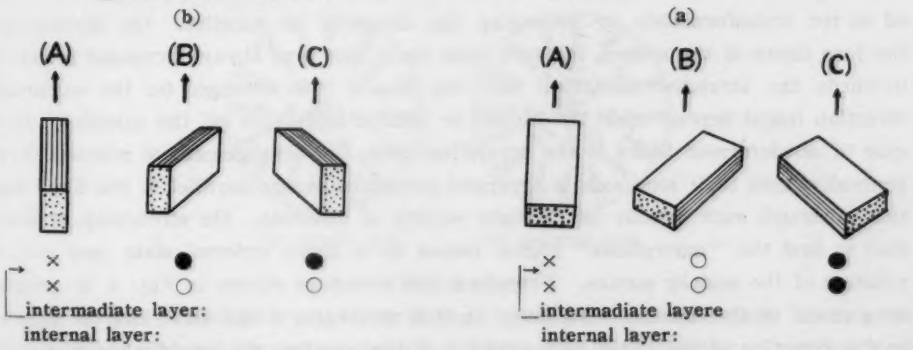
superposed over, at the (B) position, the interference color was lowered at the concave sides of hyperbolas, and was raised at the convex sides of hyperbolas but at the (C) position the correlation was reverse to the abovementioned result. (Fig. 3) From these results obtained, the fibers of the crystalline lens are considered as optically biaxial.

The interference figures of the crystalline lens consist of two or four different isogyres kinds of biaxial types. In the cortexes of the simple and complex types, the ellipsoid axis Z is normal to the lens surface, the Y-axis lies in the fibers direction, and the X-axis is right-angled to them. On the contrary, in the outer part of the nucleus of complex type, the Z-axis lies in the fibers direction, the Y-axis is normal to the lens surface, and the X-axis is at right angles to the Z-and Y-axis.

4. Effect of Deformation on Optical Characteristics of Isolated Crystalline Lens Fibers.

A strip of lens fibers excised from the cortex was examined between crossed polars with parallel polarized light. When a sensitive tint plate is inserted into the direction of fast ray (X′) paralleled with the fibers direction, the interference color was raised to blue. When the strip was stretched to the fibers direction the interference color was raised more. On the contrary, when the strip was compressed to the fibers direction, it changed from blue to yellow. The vibrating direction of slow ray (Z′) in the strip paralleled to the fiber direction was changed to the direction of deformation force by compressing. When the deforming force was released, the interference color returned to the original condition. From the results obtained by further similar experiments on every direction, Z-axis rotated to the stretched direction and X-axis rotated to the compressed direction, independently of the fibers direction.

Fig. 2. Orthoscopic observation on lens fibers
(a) normal direction to lens surface parallel to microscopeaxis
(b) normal direction to lens surface and fibers direction parpendicular to microscopeaxis
(c) fibers direction parallel to microscopeaxis
○: a rise in interference color ×: interference color of gypsusm plate itaelf
●: a fall in interference color



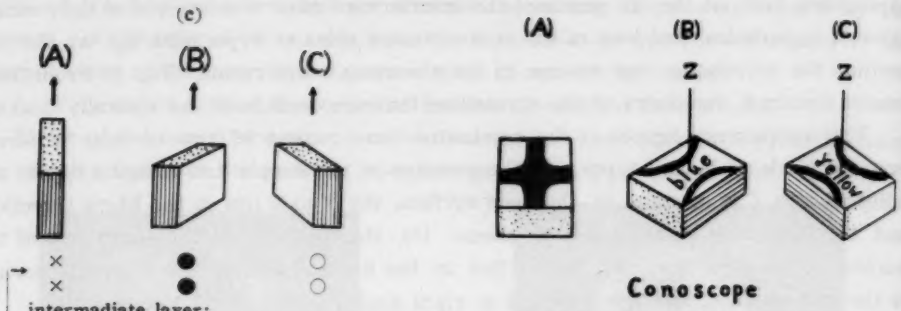
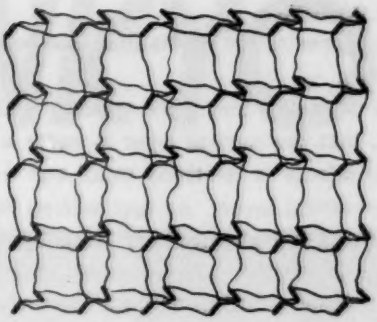


Fig. 3. Conoscopic observation on lens fibers. normal direction to lens surface parallel to microscopeaxis

Discussions

In the case of high molecular weight substances like protein, the submicroscopic structure is considered that chain molecules form "crystalline" region in a part by well-ordered arrangement and in another part form an amorphou sregion by the dis-ordered arrangement. This is, the socalled "fringe micelles" structure mentioned by Herrmann, Gerngross, Abitz, et. al. The "fringe micelles" have very important significance for understanding the various peculiar physical and chemical properties of the high mrlecular weight substances. The fringe-like "amorphous" region is an orizin of rubber-like elasticity of long chain molecules and the birefringence phenomenon appeares as the result of aggregation of micelles having double refracting action. The crystalline lens has a small water content in the nucleus and it is hard, has little elastisity, and strong birefringence. On the contrary, in the cortex it is soft and has much rubber-like elasticity and the water content is large. From these results, it is supposed, that the "crystallins" regione occupy a larger part in the nucleus of the crystalline lens, and the "amorphous" regions are increased an approaching to the cortex and the submicroscopic structure shows a kind of swelling condition. The change in birefringence observed in the case of deformation of the swollen specimens is considered as the transformation of arranging the direction of micelles. On deforming of the lens fibers of the cortex, the fact, that the Z-axis was always arranged selectively to-wards the stretched direction, and the X-axis was arranged for the compressed direction might depend upon the formal or energetic factors of the micelles. In the case of nondeformed fibers of the crystalline lens, the arrangement of micelles showed generally, that their long axis is arranged perpendicular or parallel to the fiber direction, although each micelle has a slight variety of direction. On stretching, it seems, that at first the "amorphous" region comes to a more ordered state and next the rotation of the micelle occurs. Therefore, the structure shown in Fig. 4 is proposed as a model of the micellar structure. In this structure, if the stretching is operated to the direction of the initial arrangement of the micelles, the variety of the micellar

Fig. 4. Model of micellar structure.



direction should become smaller and better ordered. On the other hand, if the streth-ing is operated perpendicularly to-wards the direction of the initial micellar arran-gement, the variety should increase and the optical property should come to be isotropic macroscopically for an anisotropic of each micelle is counteracting each other, and then, the micelles should be arranged to the stretched direction and the variety should become gradually smaller. Therefore, if such a change of micellar arranging direction caused also the differnce of birefringence characteristics in each layer of the crystalline lens, its arrangement would be shown in the following figure. (Fig. 5).

The change of birefringence was observed in the lens fibers of cortex not only by the deformation by the deforming force but also by the drying, but it is not clear that the difference between the optical properties of each layer of the crystalline lens is based on the difference of the water content.

Fig. 5. Micellaris arranging direction.

the simple type the complex type

Summary

The optical polarization properties of the crystalline lens have been investigated with both parallel and convergent polarized lights. The results obtained are as follows.

(1) From the optical polarization view point, no difference was fundameatally observed, whether of crystalline lenses of fish, nor of mammalia.

(2) Two types, such as simple and complex were observed by the investigation of the optical-polarization properties.

(3) The interference figures of the crystalline lens was found to consist of two or four biaxial type isogyres.

(4) The fibers of the crystaline lens were optically biaxial.

(5) It was presumed, that the cortical layer is under a slightly swollen condition, so that, the volume of the micelle is relatively small and this fact is induced by the weak birefringence of the cortical layer. As approaching to the nucleus, the volume of the micelle is increased and the magnitude of birefringence is also increased.

(6) A schematic diagram of the submicroscopic structure of the crystalline lens is presented for explaining their optical-polarization behavior.

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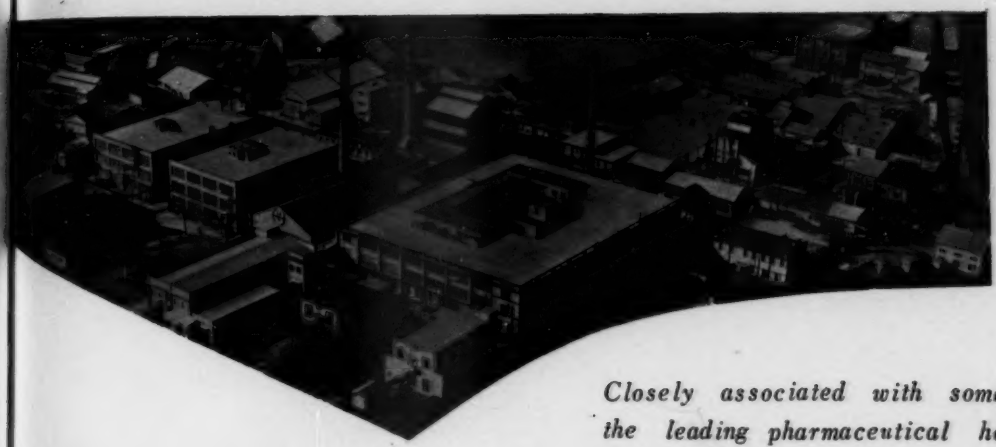

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